Review

The effects of disease modifying anti-rheumatic drugs on osteoclastogenesis and bone destruction in rheumatoid arthritis

Yuki Nanke, Toru Yago and Shigeru Kotake
Institute of Rheumatology, Tokyo Women’s Medical University

(Received June 20, 2011)

Summary

Finding the means to ameliorate and prevent bone destruction as well as control inflammation is an urgent issue in the treatment of rheumatoid arthritis (RA). Recently, it has been demonstrated that osteoclastogenesis plays an important role in the bone destruction and pathogenesis of RA. Here, we review the effects of disease modifying anti-rheumatic drugs (DMARD) on the amelioration of bone destruction and osteoclastogenesis.

Key words—synovitis; inflammation; osteoclast; receptor activator of NF-κB (RANK); receptor activator of NF-κB ligand (RANKL)

Introduction

RA is a chronic inflammatory disease characterized by synovitis and destruction of both articular cartilage and bone. Enzymatic breakdown of extracellular matrix components by matrix metalloproteinases and cathepsin has been demonstrated to play a role in cartilage destruction and bone loss. Monocyte/macrophage-derived cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and IL-6, and the T cell-derived cytokine, IL-17, are involved in the induction of osteoclasts and synovitis in RA. Cytokine-mediated osteoclastogenesis and inflammation occurs in joints and synovial tissue.

Osteoclastogenesis in RA is regulated by mechanisms, dependent on and independent of inflammation. In fact, we recently reported that the amount of T cells expressing both interferon-γ (IFN-γ) and receptor activator of NF-κB ligand (RANKL) in the peripheral blood of patients with RA does not correlate with the serum level of C-reactive protein. Since 2008, we have emphasized differences in osteoclastogenesis between humans and mice, suggesting the novel term ‘human osteoclastology’.

Disease-modifying antirheumatic drugs (DMARD) have been used to treat RA and have contributed considerably to the amelioration of both inflammation and bone destruction. There has been a growing emphasis on early diagnosis and early intensive treatment for RA due to the recognition that disability and bone damage progress rapidly during the first several years after disease onset. This early time period is considered ‘a window of opportunity’ and the early therapeutic approach has become more intensive. Recently, the goals of therapy are to cure the disease rather than achieve remission. DMARD administration is central to treat RA since it recovers the clinical signs and symptoms of RA and retards radiographic progression of joint damage. The biologics are designed to specifically target pathogenic mediators of inflammation and bone damage and are considered DMARD.

In this article, we review the effect of DMARD on osteoclastogenesis to clarify the cellular and molecular basis for their efficacy against bone destruction. We showed that not only biologics, but also DMARD could effectively repair bone destruction. In addition, we introduce some combination therapies of DMARD whose show clinical effect for bone destruction.

However, we will not review the biologics in this article. According to this review, we can know, not only biologic agents, but also other DMARD ameliorate, prevent and improve bone destruction due to RA. Since D-penicillamine (D-PC) and actarit have not been reported to show an effect on osteoclastogenesis, in this review, we will not discuss these agents.
A systemic review of randomized placebo-controlled trials:

Jones et al\(^{10}\) reviewed 38 randomized placebo-controlled trials to assess and rank the efficacy of pharmacological interventions in preventing radiological progression of RA. The two outcome measures were the weighted standardized mean difference and the progression of X-ray scores pooled as close to 12 months. According to these data, infliximab, cyclosporine, SSZ, LFE, MTX GST, corticosteroid, AF, and interleukin 1 receptor antagonist (IL-1R\(\alpha\)) were significantly better than placebo in terms of change in erosion scores (Table 1). These agents decreased the radiological progression of RA.

**Methotrexate (MTX):**

MTX mainly suppresses the metabolism of folic acid by inhibition of the enzyme dihydrofolate reductase and other folate-dependent enzymes including thymidylate synthetase. MTX has been reported to inhibit neovascularization, neutrophil activity and adherence, IL-1 and IL-8 production by stimulated peripheral blood mononuclear cells, and TNF production by stimulated peripheral T cells. MTX is currently used as an anchor drug. Clinically, MTX demonstrated retardation of radiographic progression in a randomized placebo trial\(^9\). In vitro, MTX has been reported to inhibit the generation of CD14\(\text{(+)}\) type A synovioctye-like cells from bone marrow progenitor cells (10). MTX suppressed glycosaminoglycan and collagenase release into the culture medium from IL-1\(\beta\)-stimulated rabbit chondrocytes\(^{11}\). Neidel et al\(^{12}\) reported that MTX reduced the loss of proteoglycans from articular cartilage. Some studies have demonstrated the mechanisms underlying the bone-protective effects of MTX. May et al reported the effect of low-dose-MTX on bone metabolism in rats\(^3\). MTX inhibited osteoclast formation and also inhibited the expression of RANKL in cocultures of RA FLS and PBMCs in a dose-dependent manner and increased the secretion of OPG\(^{14}\). MTX inhibited osteoclastogenesis by acting on osteoclast precursor cells and interfering with RANKL-mediated induction of NFATc\(^{15}\). MTX also had an inhibitory effect on RANKL expression in mesenchymal cells\(^{15}\). MTX suppresses inflammatory agonists such as TNF-\(\alpha\), IL-1\(\beta\), prostaglandin D2, PGE2 and PGE2-induced IL-6 synthesis in osteoblasts\(^{16}\). However, MTX has been reported to suppress osteoblast activity\(^{17}\). We reported a patient with RA who showed radiologic repair of erosions following administration MTX\(^{18}\).

**Leflumide (LEF):**

LEF is an isoxazole derivative that is structurally unrelated to other known anti-rheumatic drugs. LEF is a prodrug that is rapidly converted into its active metabolite A77-1726. It inhibits de novo pyrimidine biosynthesis by acting on dihydrogenase (DHODH). It has shown protective effect against bone damage in animal models and in humans. It also suppresses proliferation or activation of T cells and leads to immunomodulation. LEF was introduced as an immunosuppressive drug in the transplantation of allograft and approved for the treatment of RA in 1997. In a double-blind, randomized trial\(^9\), LEF showed effects similar to MTX and SSZ. In vitro, LEF has a direct inhibitory effect on RANKL-mediated osteoclast differentiation by inhibiting the induction of NFATc\(^{20}\). LEF directly and intrinsically inhibited the differentiation and function of osteoclast lineage cells without any mediation by other cells\(^{21}\). LEF also inhibits the production of prostaglandin E2, MMP-1 and IL-6 in human FLS\(^{22}\).

Radiographic analysis demonstrated less disease progression with LEF, SSZ and MTX therapy compared to that with placebo\(^{23-27}\).

**Salazosulfapyridine (SSZ):**

SSZ was initially designed as a drug that linked an antibiotic, sulfapyridine, with an anti-inflammatory agent, 5-aminosacrylic acid (5-ASA). SSZ suppresses various functions of lymphocytes and leukocytes and inhibits the enzyme 5-aminomimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, resulting in extracellular adenosine release\(^{28}\). In a randomized, double-blinded, placebo-controlled trial, SSZ has been shown to reduce the development of joint damage\(^{29}\). Lee et al reported that SSZ inhibits osteoclast formation in cocultures of RA fibroblast like cells (FLS) and peripherally modulat-

Table 1.

<table>
<thead>
<tr>
<th>DMARD</th>
<th>SMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSZ</td>
<td>-0.50</td>
</tr>
<tr>
<td>LEF</td>
<td>-0.49</td>
</tr>
<tr>
<td>MTX</td>
<td>-0.36</td>
</tr>
<tr>
<td>GST</td>
<td>-0.33</td>
</tr>
<tr>
<td>AF</td>
<td>-0.30</td>
</tr>
<tr>
<td>HAQ</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Efficacy of therapy for preventing radiological progression in rheumatoid arthritis. Summary data are presented as the weighted SMD and 95% confidence interval.
ing the RANKL-RANK-OPG interaction, primarily by decreasing expression of RANKL and increasing expression of OPG\textsuperscript{30}. SSZ was also reported to inhibit osteoclastogenesis by acting on osteoclast precursor cells and interfering with RANKL-mediated induction of NFATc1\textsuperscript{15}. SSZ strongly inhibits the mRNA expression of RANKL on osteoblasts\textsuperscript{35}. Nose et al reported that SSZ suppressed glycosaminoglycan (GAG) and MMP-1 (collagenase) release into the culture medium from IL-1β stimulated rabbit chondrocytes in a dose-dependent manner, and suppressed stromelysin (MMP-3) and PGE\textsubscript{2} release\textsuperscript{11}. Taken together, SSZ suppresses bone destruction by inhibiting both osteoclastogenesis and MMP release.

**Hydroxychloroquine (HCQ):**

The mechanism of HCQ action is not clear, although it may concentrate inside cells, principally within acidic cytoplasmic vesicles. HCQ has been reported to localize in lysosomes, and the accumulation of HCQ appears to raise the intravesical pH and may interfere with the processing of autoantigenic peptide\textsuperscript{31}. Lee et al reported that HCQ did not inhibit osteoclast formation, and that HCQ concentrations $>$ 50 m M were toxic to both FLS and PBMC. There are few data regarding the effects of HQC on bone in vitro.

**GOLD:**

There are two parenteral gold formulations, gold sodium malate and myochrysine, and an oral compound, auranofin (AF). Hall et al\textsuperscript{32} reported that gold salts dose-dependently inhibit osteoclastic bone resorption in vitro, using a bone slice assay where bovine cortical bone slices were resorbed by osteoclasts disaggregated from the long bone of neonatal rats. They also reported that AF can bind to mineralized bone surfaces and lead to decreased osteoclast survival, indicating that osteoclasts may ingest AF during bone resorption and once internalized the compound is either cytotoxic at high concentrations or inhibits the cellular process essential for bone resorption to occur at lower concentrations.

**Mizoribine (MZR):**

MZR (4-carbamoyl-1-b-D-ribofuranosylimidazolium-5-0late, MZR) is a novel immunosuppressive agent that was first isolated from culture medium of a mold, eupenicillium brefeldianum M-2166 and developed in Japan\textsuperscript{35}. It acts via selective inhibition of inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, which results in the inhibition of T cell and B cell proliferation. It suppresses collagen-induced arthritis and bone damage in mice\textsuperscript{34} and inhibits the expression of matrix metalloproteinase-1 (MMP-1) in synovial fibroblasts and THP-1 macrophages\textsuperscript{14}. MZR has been used in patients with renal transplants, lupus nephritis, nephritic syndrome and RA\textsuperscript{35}. MZR induces apoptotic death of human Jurkat T cells\textsuperscript{36} and inhibits basal bone resorption in mouse calvaria\textsuperscript{37} as well as the proliferation of rheumatoid synovial fibroblasts\textsuperscript{38}. Lee et al reported that MZR directly inhibited osteoclastogenesis in vitro in a dose-dependent manner\textsuperscript{39}. However, MZR did not affect the phosphorylation of mitogen-activated protein (MAP) kinase (p38, JNK, ERK), the degradation of IκBα or receptor activator of NF-κB ligand (RANKL) expression in fibroblast-like synoviocytes stimulated with IL-1b. Thus, MZR may inhibit the growth of precursor cells\textsuperscript{30} and downregulate the expression of genes such as RANK of precursor cells\textsuperscript{39}.

**Tacrolimus (TAC):**

TAC is an immunosuppressive agent developed in Japan in 1984 and is employed in clinical organ transplantation. TAC specifically suppresses T cell activation. It exerts its immunosuppressive effects after binding to intracellular proteins, termed FK506 binding proteins (FKBPs). This complex inhibits calcineurin phosphatase, which is involved in activation of the transcription factor NFAT, required for the expression of cytokine genes in T cells. TAC potently suppresses TNF-α and IL-1β production through T cell activation in human peripheral blood mononuclear cells. Recently, TAC was defined as a new type of nonbiologic disease-modify anti rheumatic drug in patients with RA. In vitro studies have provided some controversial data regarding osteoclastogenesis. The efficacy and safety of TAC in the treatment of RA have been reported from several countries\textsuperscript{40,41}. TAC inhibits RANKL-induced osteoclast differentiation\textsuperscript{42,43} and RANK-induced nuclear translocation and enhanced expression of NFATc1\textsuperscript{44,45}.

We reported that TAC dose-dependently inhibited dexamethasone-induced osteoclastogenesis from human PBMC by reducing the production of IL-17\textsuperscript{46}. In contrast, TAC stimulates mesenchymal cells and osteoprogenitor cells to undergo osteoblastic differentiation through a signal transduction pathway via calcineurin, resulting in excellent in vivo bone
Bucillamine (BU):

BU, N-(2-mercapto-2-methylpropionyl)-L-cysteine was developed in Japan and is widely used as a DMARD. BU is a cysteine derivative with two SH groups, and a homolog of D-PC. The actions of BU on the immune system include reinforcing suppresser T cells, suppressing both the adhesion of T cells to human vascular endothelial cells, and the proliferation of T cells. In addition, BU also affects B cells; it suppresses the production of antibodies against B cells. The main metabolites include SA679 with a monomethyl body, SA672 with a dimethyl body, and SA981 with intermolecular bonds. BU suppresses the proliferation of synovial cells in RA patients, and the reproduction of IL-1 and IL-6 in those cells in a dose-dependent manner. It has been reported that in vitro BU has bone-protective effects: BU inhibited osteoclastogenesis by acting on osteoclast precursor cells and interfering with RANKL-mediated induction of NFATc1. Previously, we reported three patients with RA who showed radiographic repair of erosions and cysts following BU treatment, supporting the in vitro data.

Combination therapy

Some combination therapies have been reported to be effective for bone destruction (Table 2). MTX is combined effectively with most other DMARD.

1. MTX + LEF

Weibatt et al. reported the pharmacokinetics, safety, and efficacy of a combination treatment with MTX and LFE in patients with active RA. Kremer et al. also reported the efficacy of a combination with MTX and LEF based on a randomized, double-blind, placebo-controlled trial.

2. MTX + SSZ

Dougados et al. reported that combination therapy with SSZ and MTX in early RA. In this randomized, controlled, double-blind clinical trial, the disease activity score (DAS) showed a significant difference after combination treatment compared with that after treatment with single components. Capell et al also showed the effectiveness of combination treatment with SSZ and MTX.

3. MTX + SSZ + HCQ

O'Dell et al reported the efficacy of a combination of SSZ, MTX and HCQ.

4. MTX + MZR

Nishimura reported a 2-year open-comparison, multicenter trial of MTX plus MZR to clarify the effect on radiological progression as well as the safety of combination MTX and MZR therapy in RA. The combination MTX and MZR therapy showed stronger prevention of joint destruction than MTX monotherapy. Kasama et al. also reported the effectiveness of low-dose pulse therapy in combination with MTX in RA patients showing an insufficient response to MTX alone.

5. MTX + TAC

Morita et al reported the efficacy of combination therapy with MTX and TAC in a retrospective study. Even when administered at a low dose (<1.5 mg/day) of TAC compared to that of MTX, the combination was highly efficacious as shown by DAS28 and EULAR response criteria.

6. MTX + BU

Ichikawa et al reported that the combination of MTX and BU has more beneficial effects on RA than MTX alone in a multicenter, double-blind, randomized controlled study. Hirohata et al. reported that the intramolecular form of BU significantly suppressed the generation of fibroblast-like cells from RA bone marrow CD34+ cells and also suppressed the production of MMP-1 (type B FLS) and VEGF. Since MTX influences type A synoviocytes, these data provide a rationale for the combination of BU and MTX to treat RA. Suematsu et al. reported that a
low dose of MTX alone had a limited suppressive effect on osteoclastogenesis, but combination with BU or SSZ increased the inhibitory effect demonstrated in an in vitro study. These reports support the efficacy of combination therapy with MTX and BU or MTX and SSZ.

7. **GOLD + BU**

Yasuda et al reported the efficacy of combination therapy with GOLD and BU\(^6\)^.

**Conclusions**

Current therapeutic strategies for RA not only ameliorate inflammation but also prevent or decrease bone destruction. Many studies showed that DMARD clinically retard radiographic progression of joint damage. This review showed that DMARD inhibit osteoclastogenesis in vitro. Clinical, structural, and functional remission is the ultimate goal of therapy for RA. Using DMARD monotherapy or combination therapy with multiple DMARD or with biologics could lead to a cure for RA.

**References**


