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REVIEW ARTICLE

Potential Role of Rebamipide in Osteoclast Differentiation and Mandibular Condylar Cartilage Homeostasis

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Abstract: ***Background*:** Temporomandibular joint osteoarthritis (TMJ-OA) is a degenerative disease that involves changes in subchondral bone and progressive degradation of cartilage. Currently, rebamipide, a gastroprotective drug, is administered to protect gastric mucosa and accelerate ulcer healing.

***Objectives*:** Recent studies have shown that rebamipide also attenuates cartilage degeneration by suppressing oxidative damage and inducing homeostasis of the extracellular matrix of articular chondrocytes. Regarding the latter, reduced expression of cathepsin K, NFATc1, c-Src, and integrin β_3, and increased expression of nuclear factor-kappa B, have been found to be mediated by the transcription factor, receptor activator of nuclear factor kappa-B ligand (RANKL).

***Methods*:** Treatment with rebamipide was also found to activate, mitogen-activated protein kinases such as p38, ERK, and JNK to reduce osteoclast differentiation. Taken together, these results strongly indicate that rebamipide mediates inhibitory effects on cartilage degradation and osteoclastogenesis in TMJ-OA.

***Results and Conclusion*:** Here, we highlight recent evidence regarding the potential for rebamipide to affect osteoclast differentiation and TMJ-OA pathogenesis. We also discuss the potential role of rebamipide to serve as a new strategy for the treatment of TMJ-OA.

Keywords: Rebamipide, osteoclast differentiation, ROS, chondrocyte, TMJ-OA, mitogen-activated.

1. INTRODUCTION

Temporomandibular joint osteoarthritis (TMJ-OA) is a degenerative disease that reflects both non-inflammatory and inflammatory changes. TMJ-OA may involve all TMJ tissues and leads to anatomical changes [1, 2]. It is characterized by chronic inflammation in synovial tissue, progressive cartilage destruction and deterioration, and subchondral bone remodeling. The etiology is associated with multiple risk factors, complex, and sometimes unknown. However, the exact pathogenesis of TMJ-OA remains unclear and controversial. Osteoarthritis (OA) often affects the TMJ of patients with temporomandibular disorders (TMDs). Thus, TMJ-OA is an important subtype in the classification of TMDs [1, 3-5].

Patients with TMJ-OA often have pain and TMJ dysfunction with decreased quality of life [6]. The clinical signs and symptoms of TMJ-OA include a restriction in joint function and severe pain due to the presence of synovitis [3]. The pain usually associated with limitation of joint opening, stiffness, and may be relieved with rest and nonsteroid anti-inflammatory drug (NSAID) treatment [6]. Drug repositioning or reprofiling has a significant advantage over traditional drug development because a repositioned drug has already completed toxicity and safety tests and exhibited reduced toxicology. Investigations of new pleiotropic effects of drugs are very valuable and can enhance the success of pharmaceutical companies [7].

Rebamipide is an amino acid analog of 2 (1H)-quinolone and is widely used as a gastroprotective drug to treat gastric ulcers and gastritis. Rebamipide has also exhibited antibacterial effects, mucin secretagogue activity, and anti-inflammatory actions [7-11]. In endothelial inflammation, rebamipide suppresses interleukin (IL)-8 production through inhibition of IκBα phosphorylation and nuclear factor-kappa B (NF-κB) p65 [12]. Rebamipide also inhibits T cells activation, suppresses Th1 cytokines (IL-2 and interferon-γ), serum autoantibodies, IgM, and IgG1 production, and decreases NF-κB activity in autoimmune lesions of salivary glands [13].

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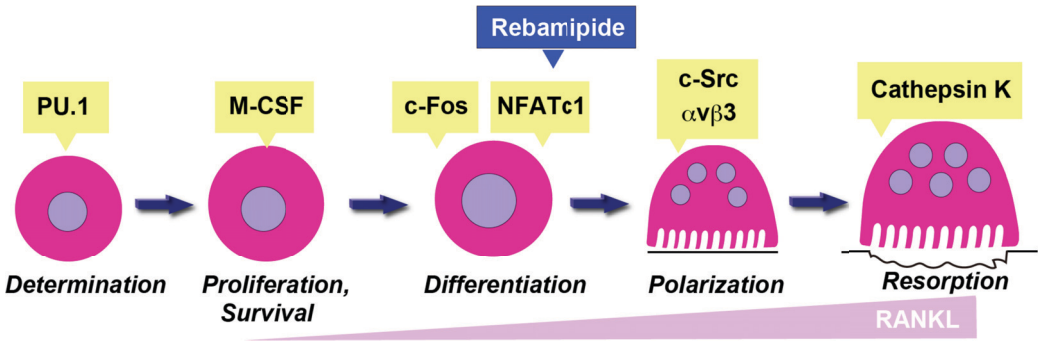

Fig. (1). Rebamipide affected osteoclastogenesis. Early nonspecific differentiation of OCs, survival cytokine M-CSF, and macrophage proliferation are dependent on PU.1. Subsequently, RANKL-induced activation of RANK commits OCs to differentiation *via* c-Fos and NFATc1. Polarization, which requires c-Src and $\alpha_v\beta_3$ integrin, is the first step in establishing the resorptive capacity of mature OCs. Osteoclasts then mobilize the mineralized component of bone, with cathepsin K mediating degradation of the organic matrix of bone. In the present study, rebamipide treatment decreased RANKL-induced NFATc1 signaling and levels of c-Src and $\alpha_v\beta_3$ integrin. As a result, expression of cathepsin K was affected.

Moreover, recent studies have demonstrated the treatment effects of rebamipide for new indications such as TMJ-OA and its potency to inhibit the formation of human osteoclasts (OCs) by inhibition of RANKL-mediated osteoclastogenesis, disruption of actin ring formation, and reduction of DC-specific transmembrane protein (DC-STAMP) [7, 14]. In OA cartilage, rebamipide attenuates matrix metalloproteinase (MMP)-13, IL-1β, hypoxia inducible factor (HIF)-2α, inducible nitric oxide synthase (iNOS), and nitrotyrosine [15]. Most of the studies suggest that rebamipide might be a potential therapeutic strategy for OA.

Bone is a tissue that is continuously remodeled via two distinct processes, bone resorption and bone formation [16]. To maintain skeletal homeostasis, these events are tightly regulated and strongly linked [17]. OCs are critical for both processes and they include bone resorbing cells which represent differentiated cells that derive from hematopoietic cells of monocyte-macrophage lineages. Conversely, osteoblasts (OBs), which derive from mesenchymal origins, are responsible for bone forming cells. When the differentiation/activity of OCs and OBs is altered, bone diseases can develop. Moreover, accumulating evidence supports the existence of a relationship between bone and the immune system, particularly in relation to pathological conditions in which activation of bone adsorption and bone resorption occurs [18].

RANK is expressed by OC progenitor cells and its activation by RANKL results in the downstream stimulation of factors associated with tumor necrotic factor (TNF) receptor, as well as several signaling cascades including mitogen-activated protein kinase (MAPK), NF-κB, activating protein 1 (AP-1), and the transcription factor, nuclear factor of activated T-cells c1 (NFATc1). Cross-talk between these signaling proteins results in the formation of OCs that are multinucleated and exhibit bone resorbing activity [19, 20]. Terminal differentiation of OCs is characterized by expression of c-Src, integrin β_3, cathepsin K, NFATc1, and other markers of OC differentiation [20, 21] (Fig. **1**). In this review, the effect of rebamipide on the differentiation and function of OCs both *in vitro* and *in vivo* is described in an effort to develop a novel strategy to treat OC-associated bone diseases.

2. TMJ-OA

OA is the most common degenerative joint disorder that causes disability in the adult population [22]. OA patients suffer from pain during increased function and load bearing joint. The joints become tender, with decreased range of motion, and loss of articular cartilage indicated by crepitus. Radiography may show joint space narrowing, formation of osteophytes [23, 24], subchondral bone cysts, condylar head flattening, and increased subchondral cortical thickness [25, 26].

TMJ is frequently affected by OA [27, 28]. The prevalence of TMJ-OA has been observed to be 25% in 20-49 year age group and 70% in 73-75 year age group by clinical and MRI examination [27, 29]. The evidence of TMJ-OA is clinically indicated by female preponderance, with a female-to-male ratio of more than 2:1 [30, 31]. TMJ-OA has various etiologies and multifactorial factors including inflammatory, immunologic insults, biomechanical, biochemical, excessive mechanical stress, and extracartilaginous factor. The extracartilaginous factors include reduction of synovial fluid, changes in synovial membrane, changes in vascular system, and subchondral bone microfractures [32, 33].

The pathogenesis of TMJ-OA can be caused by various etiologic factors that each interact with the other and each of etiologic factor may not cause the same pathogenesis. Literally, the subchondral bone has an etiological role in the pathogenesis of TMJ-OA. Failure in internal remodeling system of mandibular condylar subchondral bone may result chondrocyte injury then leads to increase collagen degradation, with release of proteases and decrease of protease inhibitor thereby resulting in extracellular matrix (ECM) breakdown [33].

Various key mediators have been suggested to be responsible for degradation of articular cartilage *in vivo* and *in vitro* including MMP-13 and members of the closely related family of a disintegrin and metalloprotease with thrombospondin motifs 5 (ADAMTS5) [34-39]. During the process of TMJ-OA, articular chondrocytes release IL-1, TNF-α, runt-related transcription factor 2 (RUNX2), alkaline phosphatase, and type 10 collagen. Concurrently, abnormal cartilage calcifica-

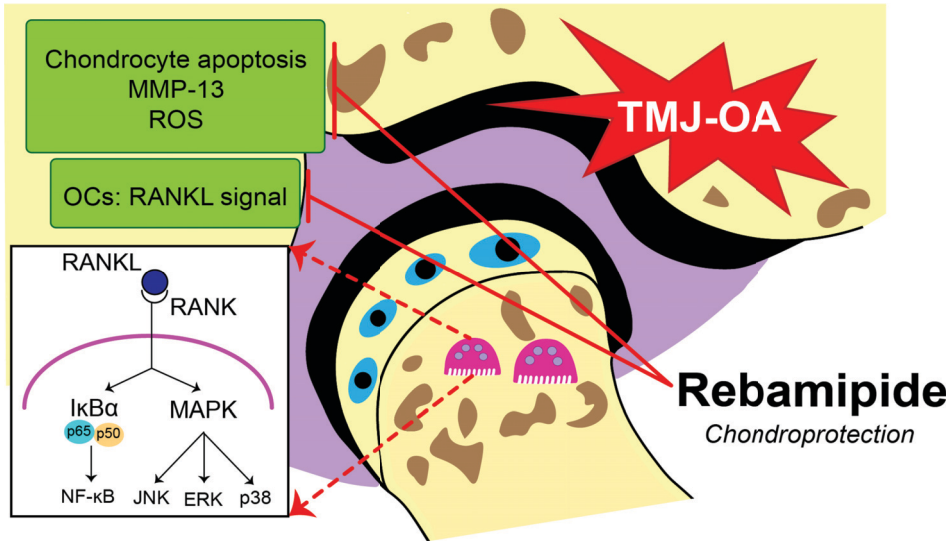

Fig. (2). Rebamipide-induced chondroprotective effects in TMJ-OA. TMJ-OA that was treated with rebamipide exhibited suppressed chondrocyte apoptosis, expression of MMP-13, and oxidative damage in a dose-dependent manner. A reduction in condylar subchondral bone volume due to blocked OC activity *via* RANKL-induced osteoclastogenesis was also readily observed.

tion occurs and exhibits decrease levels of proteoglycan [40-45].

3. REBAMIPIDE AND THE DIFFERENTIATION OF OCs

Multinucleated cells that attach to the bone matrix via an actin rich structure are referred to as OCs. The actin rings that are formed by OCs degrade bone matrix following the secretion of protons and proteases into a space that forms between OCs and a bone surface through a specialized structure known as a ruffled border membrane [46]. OC differentiation is regulated by colony stimulating factor 1 receptor (Csf1r), also known as M-CSF receptor or c-Fms, and tumor necrosis factor receptor superfamily member 11a (Tnfrsf11a), also known as receptor activator of NF-κB (RANK) [47]. While M-CSF receptor signaling supports the survival and proliferation of OC precursor cells during osteoclastogenesis, the differentiation process of OCs is activated by RANK signaling. RANK signaling activates NF-κB and Fos, both of which are transcription factors that are essential for OC differentiation [21]. Ig-like receptors transmit signals to activate phospholipase Cγ (PLCγ) via their adaptors, DAP12 and FcRγ, with each containing an immunoreceptor tyrosine-based activation motif (ITAM) [48]. PLCγ then induces calcium oscillation, which leads to the activation of calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase. RANK and Ig-like receptor signals are finally integrated by the master transcription factor of osteoclastogenesis, NFATc1, which induces the expression of molecules that allow OCs to perform bone resorbing activities. These molecules include MMP-9, cathepsin K, the chloride channel, CLC-7, and H^+-ATPase subunits [49].

Rebamipide treatment of bone marrow macrophages (BMMs) and human monocytes inhibits RANKL-induced OC formation from precursor cells in a dose-dependent manner without cytotoxicity. Thus, rebamipide affects the generation of OCs from macrophage that are stimulated with RANKL and also the differentiation of OCs [7, 14].

4. REBAMIPIDE SUPPRESSES GENE EXPRESSION IN OCs

Previous studies have reported that rebamipide inhibits IL-8 expression that is induced by TNF-α by suppressing NF-κB signaling in human umbilical vein endothelial cells (HUVECs). Rebamipide also inhibits the adhesion of endothelial cells to endothelial cells that are stimulated by hypoxia/reoxygenation through an NF-κB-dependent pathway [12, 50]. For RANKL-induced OC differentiation, the NF-κB pathway must be activated [49]. Activation of the IκB kinase complex is a well-characterized aspect of the classical NF-κB signaling pathway, and it leads to phosphorylation of IκBα which targets it for ubiquitin-dependent degradation [51]. We have shown that rebamipide inhibits the degradation of IκBα in the cytoplasm to reduce transactivation of NF-κB [14].

It has been reported that MAPKs (*e.g.* p38, JNK, and ERK) are activated by RANKL stimulation and they play a role in osteoclastogenesis [19]. In the early stages of OC generation, p38 is particularly important based on its ability to regulate microphthalmia-associated transcription factor [52]. Meanwhile, dominant-negative JNK has been shown to prevent osteoclastogenesis induced by RANKL [53]. ERK is able to induce c-Fos for osteoclastogenesis [54], while inhibition of ERK reduces OC formation [55]. In the present study, activation of these MAPKs was investigated following rebamipide treatment, and it was observed that rebamipide inhibits the phosphorylation of each. Thus, the anti-osteoclastogenic effect of rebamipide in RANKL-stimulated BMMs may be mediated via the phosphorylation of various MAPKs [20] (Fig. **2**).

5. REBAMIPIDE DISRUPTS THE CYTOSKELETAL ORGANIZATION OF OCs TO INHIBIT BONE-RESORBING ACTIVITY

Degradation of the inorganic and organic matrices of bone is a crucial function of OCs. The accumulation of molecules that are able to degrade bone on the resorption

surface of bone requires direct interactions between OCs and mineralized substrates. OCs create a microenvironment that is isolated from the extracellular space by restructuring the actin component of their cytoskeleton. Specifically, actin rings are formed that provide a "gasket-like" sealing of this microenvironment [56].

In the present study, assays to detect pit formation induced by RANKL show that rebamipide inhibits the ability of OCs to perform bone-resorbing activities. In addition, rebamipide treatment leads to the degradation of actin rings of mature OCs in a dose-dependent manner. However, following the exposure of bone marrow stromal cells to rebamipide, β-glycerophosphate, and osteoblastogenic medium, the mineralization and differentiation of the OCs were unaffected. Thus, rebamipide appears to mediate an anti-resorption effect, while indirectly affecting the formation of bone [14].

6. MURINE MODEL OF TMDs

As a synovial joint, the TMJ is essential for sliding and hinge movements of jaws [57]. The TMJ consists of the condyle, articular eminence, and glenoid fossa, and it provides articulation between the mandible and the cranium. These joints are surrounded by a capsule consisting of a synovial lining and fibrous material and they provide anatomic control of both occlusion and mandibular movement. An articular disk separates the joint space between the condyle and the glenoid fossa into lower and upper articular cavities and these are bounded by the condyle and the articular eminence and articular fossa, respectively [58, 59].

TMDs include a heterogenous cluster of diseases [60-62]. In particular, rheumatoid arthritis (RA) and OA of the TMJ represent severe and debilitating disorders whereby the TMJ disk can undergo displacement, thickening, folding, lengthening, and disk perforation [63-65]. However, the factors responsible for the development and progression of TMD, especially OA in the TMJ, remain to be determined [57].

As compared to articular cartilage in the knee, mandibular condyle cartilage is considered secondary (*e.g.* from the chondroskeleton) and it derives from the cranial neural crest during embryogenesis. Compared to other synovial joints and the articular cartilage of other joints, the condyle of the mandible has less type I collagen and does not express type II collagen in the superficial layer of mandibular condylar cartilage. In addition, the articular surfaces of the mandible are composed of fibrous tissue rather than hyaline cartilage. For studies of TMDs, specific devices and methods have been applied to establish mouse and rat models of these diseases. In addition, various genetic animal models of OA have been established [66].

The TMJ-OA model established in the present study exhibited irregularities in chondrocyte alignment in the condylar cartilage layers, OA-like degenerated lesions, marked depletion of proteoglycans, and subchondral bone loss due to OC hyperactivity. Previously, it was demonstrated that forced mouth opening in mice led to a decrease in subchondral bone volume [67], while steady and repetitive jaw opening was an effective method for inducing OA-like changes in rabbits. Moreover, the latter were consistent with the presentation of TMJ-OA in patients [68]. Biomechanical stimulation from abnormal occlusion [69, 70], local application of chemicals [3], surgical manipulation of the joint [71], and genetic modifications [72, 73] have also been shown to induce early TMJ-OA.

7. REBAMIPIDE AND AUTOIMMUNITY

In a recent study, adjunct rebamipide therapy was found to effectively prevent peptic ulcers in patients that were prescribed a COX-2-selective inhibitor for arthritis [74]. Oral administration of rebamipide has also been found to reduce histological and clinical scores in animal models of RA, particularly in SKG mice and animals with arthritis induced by collagen [75, 76]. RA leads to inflammation of the synovial membrane and involves the production of IL-17, IL-1β, IL-6, and TNF-α. Expression of RANKL is also enhanced in synovial cells, thereby inducing OC differentiation. Considering these results, as well as the observation that CD4+ T cell activation is suppressed by rebamipide, it is possible that rebamipide may also be useful for the healing of bone destruction that is impaired by OCs [7, 13, 77].

Decreased apoptosis of epithelial cells in the salivary glands has been observed in rebamipide-treated mice. Rebamipide treatment has also been found to suppress the activation of Th1 cytokines (IL-2, interferon-γ) and CD4+ T-cells, thereby adversely affecting NF-κB activity and inhibiting the expression of IRF-4B, a transcription factor associated with B-cell activation and differentiation. Thus, rebamipide may represent a novel therapeutic approach for Sjorgen syndrome [13].

8. THE ROS-SCAVENGING PROPERTY OF REBAMIPIDE AND TMJ-OA

Active oxygen species that are generated by polymorphonuclear leukocytes represent a potential source of damage to cells. Meanwhile, reactive oxygen metabolites that are generated during the metabolism of arachidonic acid, smooth muscle cells, and platelet macrophages may contribute to the damage of gastric mucosa. A pharmacological effect of rebamipide includes its ability to scavenge hydroxyl radicals and attenuate the cytotoxicity of reactive oxygen metabolites. In regard to gastric mucosal damage, rebamipide has the potential to scavenge hydroxyl radicals and also suppress the production of active oxidants by modulating the activation of neutrophils [78-80].

A recent report described the inhibitory effects of rebamipide on cartilage degeneration and pain production in an experimentally induced model of OA in rat knee tissue. Oral administration of rebamipide reduced oxidative stress in the subchondral bone area and in articular cartilage. Furthermore, the chondroprotective capacity of rebamipide was associated with reduced catabolism of the articular cartilage matrix. ADAMTS5, MMPs, and HIF-2α have also been reported to mediate the ability of rebamipide to serve as an anticatabolic regulator of cartilage destruction [15].

In the present study, excessive chondrocyte apoptosis and enhanced expression of MMP-13 by chondrocytes characterized the model of TMJ-OA that was established. Then, following treatment with rebamipide, a dose-dependent attenua-

tion of cartilage degradation was observed in the hypertrophic layer of the condylar cartilage [14].

During the cartilage degradation process, reactive oxygen species (ROS) and antioxidants may simultaneously act at different levels. Both induction of matrix degradation by enzymes and inhibition of matrix formation are involved in this process. Based on the role of ROS in mediating an increase in the apoptosis of chondrocytes during OA, ROS have been identified as a potential treatment target. In addition, inhibitor of nitrite oxide, a marker of oxidative stress, has been found to be markedly attenuated in TMJ-OA mice treated with rebamipide. Thus, the chondroprotective effects of rebamipide on cartilage affected by TMJ-OA may be mediated by its ROS-scavenging property [14, 77, 81, 82] (Fig. **2**).

9. OCs AND ARTICULAR CHONDROCYTE MALFUNCTION IN TMJ-OA AND POTENTIAL EFFECTS OF REBAMIPIDE

Articular cartilage and subchondral bone are separated by a calcified cartilage zone that undergoes marked changes in structural, physical, and functional properties of the OA process [83]. OC, a multinucleated cell responsible for bone resorption, can penetrate the mineralized matrices of the bone and calcified cartilage [84].

The penetration site creates fissures and cracks in the overlying cartilage and the vascular supplies a mechanism for fluids exchange and soluble mediators between these tissues. During the OA mechanism progresses, cracks and discontinuities also develop in the subchondral bone that leads to the mechanism for exchange. There is an evidence of communication between the cartilage and subchondral bone directly via the diffusion process that allows soluble products exchange to regulate the activities of resident cells in the adjacent tissues [83, 85, 86].

A recent study reported that β-catenin signaling in chondrocytes plays an important role in postnatal bone growth and bone remodeling through OC formation in mice with conditional knockout or activation of chondrocyte-specific β-catenin [87]. A role of β-catenin is also identified in the regulation of chondrocyte differentiation and function undergo OA condition [88, 89]. Mice with β-catenin deficiency in chondrocytes exhibit increased RANKL/OPG ratios that promote OC-inducing activity. The RANKL/OPG ratio was reversed in the chondrocytes from the mice with the activating mutation, showing impaired osteoclast-inducing activity [87].

Coculture studies between chondrocyte cells and OC precursor cells with M-CSF and 1,25-dihydroxyvitamin D_3 exhibited that the ability of the chondrocytes to support osteoclastogenesis could be attributed to their differential capacity to express RANKL and OPG. OPG acts as a key inhibitor of OC differentiation via interaction with RANKL, by antagonizing the function of RANKL [90, 91]. However, in addition to osteocytes and other OB lineage cells that promote RANKL and OPG expression, chondrocytes may serve as a potential regulator in osteoclastogenesis. Thus, further studies are needed to understand the potential role of chondrocytes-derived RANKL/OPG in the pathogenesis of OA [91].

This present study demonstrated that in murine TMJ-OA, rebamipide effectively attenuates the subchondral trabecular bone resorption by decreasing number of TRAP-positive cells. In OC differentiation process, rebamipide inhibits NFATc1 [14], a transcription factor that most potently induced by RANKL [49], followed by lower levels of OC function markers such as, integrin β_3, c-Src, and cathepsin K as well as disrupted actin ring formations (Fig. **1**). Rebamipide was also found to significantly inhibit the phosphorylation of IκBα, JNK, ERK, and p38 [14]. Therefore, rebamipide has potential effects to overcome osteoclastogenesis malfunction in murine TMJ-OA (Fig. **2**).

Cellular interaction in crosstalk *in vitro* between chondrocytes and OBs has been investigated. OBs and articular chondrocytes derived from OA bone exhibit decreased production of aggrecan and chondrocyte markers (SOX9 and type 2 collagen), but increased the production of MMP-3 and MMP-13. This finding suggests that local factors expressed by OB initiate chondrocyte hypertrophy and matrix mineralization [92-94]. In murine TMJ-OA, increased expression of MMP-13 and excessive chondrocyte apoptosis was attenuated by treatment with rebamipide. However, in *in vitro* study, rebamipide was neither affected OB mineralization nor differentiation [14]. Thus, following investigations of rebamipide are needed to provide an effective treatment for TMJ-OA patients.

CONCLUSION

In the hypertrophic layer of condylar cartilage, rebamipide-treated TMJ-OA joints underwent marked degradation of cartilage, excessive chondrocyte apoptosis, and increased expression of MMP-13 by chondrocytes in a dose-dependent manner compared to vehicle-treated TMJ-OA joints. To further elucidate how these changes affect the homeostasis of cartilage ECM and induce chondroprotection, and to determine whether rebamipide affects the survival of OA chondrocytes, additional studies are needed.

Differentiation and OC activity in the rebamipide-treated TMJ-OA joints were suppressed, while highly effective antiresorptive activity was observed, as a result of inhibited transcription factor activity in response to RANKL. Thus, the present results support further study of the potential for rebamipide to serve as a treatment for TMJ-OA.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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