Pharmacological Topics of Bone Metabolism:  
A Novel Bisphosphonate for the Treatment of Periodontitis

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Abstract. It has been reported that the pharmacological characteristics of bisphosphonates vary depending on the side chain attached to the carbon atom of the P-C-P bond. TRK-530 is a novel synthetic bisphosphonate with an anti-oxidant methylthio-phenylthio side chain. This compound has been suggested to have both anti-inflammatory and anti–bone-resorbing effects. Such a compound could be effective for the treatment of diseases with excessive bone resorption accompanied by inflammation. We have been studying this compound as a potential therapeutic agent for periodontitis. To date, we have found that 1) TRK-530 inhibited osteoclastic bone resorption in animals and in bone organ culture, 2) both systemic and topical administration of TRK-530 prevented alveolar bone loss in animals with experimental periodontitis, 3) TRK-530 prevented prostaglandin E₂ synthesis by inhibiting the expression of cyclooxygenase (COX)-2 mRNA, and 4) TRK-530 inhibited the formation of dental calculus. The above results suggest that TRK-530 might be useful for the treatment of alveolar bone loss in periodontitis.

Keywords: bisphosphonate, periodontitis, bone resorption, prostaglandin E₂, dental calculus, bone metabolism

Introduction

Bisphosphonates (BPs) are pyrophosphate analogs that can suppress osteoclastic bone resorption. These compounds are used in the treatment of metabolic bone diseases that are associated with excessive bone resorption, including osteoporosis, Paget’s disease, and cancer-related diseases such as hypercalcemia, multiple myeloma, and bone metastases secondary to breast cancer and prostate cancer (1). BPs possess a P-C-P backbone, to which two side chains (R₁ and R₂) are attached. It has been reported that the pharmacological characteristics of bisphosphonates vary depending on the nature of the side chain (1 – 5).

Disodium dihydrogen-4-[(methylthio) phenylthio] methanebisphosphonate (TRK-530) is a novel synthetic bisphosphonate with an anti-oxidant methylthio-phenylthio group in the R₂ side chain (Fig. 1). This compound has been suggested to have both anti–bone-resorbing and anti-inflammatory effects (6 – 8). Such a compound could be effective for the treatment of diseases with excessive bone resorption accompanied by inflammation.

Fig. 1. Chemical structure of disodium dihydrogen-4-[(methylthio) phenylthio] methanebisphosphonate (TRK-530) and bisphosphonates.
Periodontitis is one of the most frequent diseases in dental clinics and is characterized by excessive bone resorption and inflammation caused by plaque bacteria. We have been studying TRK-530 as a potential therapeutic agent for periodontitis. This review briefly summarizes the nature of this compound.

**Inhibitory effect on bone resorption**

When administered systemically, TRK-530 can increase bone mineral density in various bones in a dose-dependent fashion. Figure 2 shows soft X-ray microangiographs of the tibial metaphysis from a normal rat and from rats treated with TRK-530 (subcutaneous daily injection for 7 days). This compound, like other bisphosphonates, can block the resorption of calcified cartilage in the growth plate, subperiosteal bone, and primary spongiosa in the metaphysis, leading to a radiologically more dense structure than normal and club-shaped tibia with a decreased marrow cavity.

In organ culture of neonatal mouse calvaria, TRK-530 can inhibit bone resorption induced by various means. In fact, the effects of all the stimulators of bone resorption tested to date, including lipopolysaccharide (LPS), prostaglandin E$_2$ (PGE$_2$), interleukin (IL)-1$\beta$, and tumor necrosis factor (TNF)-$\alpha$, which have been considered to be important causal factors of alveolar bone loss in periodontitis, have been dose-dependently prevented by TRK-530. Figure 3 shows the effect of this compound on LPS-induced bone resorption in cultured mouse calvaria. In the presence of TRK-530, bone-resorbing osteoclasts over resorption lacunae were smaller, the

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**Fig. 2.** Effect of subcutaneous daily injection of TRK-530 (0, 0.4, 2.0, or 10 mgP/kg per day) for 7 days on the bone mineral density of tibial metaphysis in rats. Soft X-ray microradiographs (left panels) and their indexed-color images (right panels).

**Fig. 3.** Laser scanning confocal microscopic images of neonatal mouse calvaria cultured for 48 h in the presence of LPS alone (10 $\mu$g/ml) (upper panels) or in combination with TRK-530 (25 $\mu$M) (lower panels). Calvaria was stained for TRACP activity. Left panels: TRACP-positive osteoclasts and right panels: Normarsky image of corresponding site in the left panel. Arrows and arrowheads indicate the edge of resorption lacunae and cytoplasmic vacuolization, respectively.
sealing zone shown by intense tartrate-resistant acid phosphatase (TRACP) staining was diffuse, and the size and depth of resorption lacunae were reduced compared with those in calvaria cultured with LPS alone. These findings suggest that TRK-530, like other bisphosphonates (9, 10), inhibits bone resorption by inhibiting the function of osteoclasts.

**Inhibitory effect on alveolar bone resorption in rats with experimental periodontitis**

Previous studies have suggested that administration of BPs is effective for preventing alveolar bone loss in experimental periodontitis (11–14). We examined whether topical administration of TRK-530 could prevent alveolar bone loss in rats with experimental periodontitis.

Elastic rings were placed around the cervix of the right and left maxillary M1 (first molar) to induce inflammatory periodontitis. Fifty microliters of TRK-530 solution (0–25 mM) was injected into the sub-peritoneal paratal area adjacent to the interdental area between M1 and M2 (second molar) on either the left or right side (control or experimental side) on day 0, 2, 4, and 6. The rats were killed on day 7. Microradiographic and histological examinations revealed that placement of the elastic ring induced severe vertical and horizontal bone resorption on the control side, while the topical administration of TRK-530 significantly prevented such alveolar bone loss on the experimental side (Fig. 4). The results suggest that administration of TRK-530 may be effective in preventing alveolar bone loss in vivo.

**Inhibitory effect on the synthesis of PGE2**

Since periodontitis is an inflammatory disease, it may be desirable to have access to a compound that could prevent inflammation in addition to bone resorption. Previous studies have shown that TRK-530 can prevent rat adjuvant arthritis that might be the result of a decrease in inflammatory cytokines such as TNF-α and neutrophil chemoattractant (CINC)-1. A decrease in serum sialic acid, a systemic parameter of inflammation, has also been reported in TRK-530–treated animals with adjuvant arthritis (7, 8). Based on these findings, we have been studying the effect of this compound on PGE2 synthesis in organ culture of neonatal mouse calvaria. Thus far, we have found that TRK-530 (0–125 µM) dose-dependently prevented a LPS-stimulated increase in PGE2 synthesis during culture (Fig. 5). The expression of cyclooxygenase (COX)-2 mRNA and COX-2 protein was also prevented. Since TRK-530 has an anti-oxidant side chain (6) and can inhibit the generation of superoxide anion that reacts with nitric oxide (NO) to form peroxynitrite (ONOO−), which is known to be a potent stimulator of COX-2 expression, the inhibi-

![Fig. 4](image_url). Inhibitory effect of topical TRK-530 on alveolar bone loss in rats with experimental periodontitis. Placement of an elastic ring around the cervix of M1 induced considerable recession of periodontal tissues: severe vertical and horizontal resorption of bone in the interdental area between M1 and M2, widening of periodontal ligament space along the roots, and irregular increase in radiolucency in the remaining alveolar bone were noted on the control side (upper panel). In the alveolar bone on the experimental side, which was injected with TRK-530 at 10 mM, these recessive changes were prevented (lower panel). M1, M2, and M3: The first, second, and third molars, respectively.

![Fig. 5](image_url). Effect of TRK-530 (0–125 µM) on the synthesis of PGE2 by neonatal mouse calvaria cultured for 72 h in the presence of LPS (10 µg/ml).
Inhibitory effect on the formation of dental calculus

It is well known that a large amount of dental calculus, especially subgingival calculus, may hamper the efficacy of oral hygiene and thereby accelerate plaque formation, the accumulation of which initiates the inflammatory reaction in periodontal tissues that leads to periodontitis. Since bisphosphonates strongly bind to calcium phosphate crystals and inhibit their growth and aggregation (1), TRK-530 may have an anti-calculogenic effect in addition to its anti–bone-resorbing and anti-inflammatory effects. Therefore, using rats that were fed a calcine diet for 2–4 weeks (15), we examined whether this compound has such an effect. As expected, TRK-530 inhibited dental calculus formation in a dose-dependent fashion when it was given in drinking water (Fig. 6) (16). However, subcutaneous injection of TRK-530, at a dose that was assumed to correspond to the maximum amount of this compound absorbed from the intestine when rats received 1.5 mM TRK-530 in drinking water, did not have any significant effect, suggesting that the anticalculogenic effect of this compound was topical rather than systemic.

Conclusion

In summary, the anti–bone-resorption, anti-inflammatory, and anti-calculogenic effects of TRK-530 suggest that if an appropriate drug-delivery system can be developed, this compound might be useful clinically as a therapeutic agent for periodontitis.

References