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COMMENTARIES

Take Two Aspirin: for Osteoporosis?

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Commentary on: Yamaza T, Miura Y, Bi Y, Liu Y, Akiyama K, Sonoyama W, Patal V, Gutkind S, Young M, Gronthos S, Le A, Wang CY, Chen W, Shi S. Pharmacologic stem cell based intervention as a new approach to osteoporosis treatment in rodents. *PLoS ONE*. 2008 Jul 9:3(7):e2615

Post-menopausal osteoporosis is characterized by increased bone turnover, with bone resorption exceeding formation, leading to loss of bone mass. Estrogen deficiency in rodents is associated with increased expression of inflammatory cytokines by lymphocytes, which is likely to contribute to bone loss. The objectives of the study by Yamaza *et al*. (1) were to investigate the role of inflammatory T cells in bone marrow mesenchymal stem cell apoptosis and in ovariectomy (OVX)-induced bone loss in mice and to examine whether aspirin could prevent bone loss and the effects of activated T cells.

This complex study comes from 5 different research centers in the United States, Japan and Australia and has two provocative messages that deserve further investigation (1). The first is an extension of prior studies on the role of T cells in the pathogenesis of osteoporosis (2), while the second is a new finding that pretreatment with aspirin may mitigate bone loss in OVX mice by a T-cell dependent mechanism. Prior studies of the role of lymphocytes in the pathogenesis of post-menopausal osteoporosis have largely focused on bone resorption (2). The first part of the manuscript describes experiments with activated T cells co-cultured with bone marrow mesenchymal stem cells (BMMSCs). Activated T cells are shown to increase apoptosis of BMMSCs through the Fas/FasL pathway, suggesting that activated T cells may decrease bone formation. The authors also examined the effect of OVX on bone mass of immunocompromised (bg-nu/nu-xid) mice that do not have T cells. They show that these mice do not lose bone mass with OVX as measured by DXA. However, DXA predominantly measures changes in cortical bone mass, and it was previously reported that T-cell deficient mice maintain their cortical bone mass (3). There are also conflicting reports on trabecular bone loss after OVX in T-cell deficient mice, perhaps due to different experimental models (3;4). In the study by Yamaza *et al*. (1) the inflammatory T-cells increased osteoclasts associated with trabeculae, suggesting that there may be loss of trabecular bone, but this loss should be confirmed with μCT or histomorphometric studies.

The authors then examined the effects on bone mass in OVX immunocompromised mice of adding back a population of pro-inflammatory T cells ($CD4^+CD25^-CD45RB^{+hi}$), which were previously shown to induce inflammatory bowel disease in immunocompromised mice. The $CD4^+CD25^-CD45RB^{+hi}$ T cells caused bone loss in OVX immunocompromised mice while the reciprocal $CD4^+CD25^-CD45RB^{-/low}$ T cells did not. It is likely that the bone loss induced in the OVX immunocompromised mice after T cell transfer resulted from production of cytokines and cellular immune mediators of inflammatory disease in these mice. Since the authors did not examine the effects of

transferring $CD4^{+}CD25^{-}CD45RB^{+hi}$ T cells into sham-operated mice, it is unclear if the role of these inflammatory T cells is specific for bone loss after OVX. It is possible that sham-operated mice would lose equal amounts of bone after transfer of $CD4^{+}CD25^{-}CD45RB^{+hi}$ T cells.

The second series of experiments with aspirin are novel. OVX mice were pretreated for 2 months with aspirin before surgery and continued on aspirin for another month after OVX. These aspirin-treated OVX mice were compared with sham-operated and OVX mice without any aspirin treatment. Aspirin-treated OVX mice had less trabecular bone loss, measured by μCT, compared to OVX only mice. However, since there were no sham-operated controls treated with aspirin, it is not clear whether the effects observed with aspirin treatment are specific to OVX. It is possible that aspirin has effects in sham-operated mice.

BMMSCs were isolated from OVX mice and aspirin-treated OVX mice and implanted into immunocompromised mice. Transplantation into immunocompromised mice of BMMSCs from aspirin-treated OVX mice formed more bone than BMMSCs from OVX mice. The authors demonstrated that aspirin can inhibit Fas-stimulated death of human BMMSCs and decrease viability of activated T cells. Using cultured human BMMSCs, they showed that aspirin can increase *in vitro* mineralization and slightly increase telomerase activity and telomere length. Human BMMSCs expanded *in vitro* and treated with aspirin before being transplanted into immunocompromised mice formed more bone compared with BMMSCs not treated with aspirin.

In addition to the effects on osteoblastic cells, there were fewer osteoclastic cells in OVX mice treated with aspirin compared with OVX alone. The decrease in osteoclastic cells in aspirin-treated OVX mice was associated with decreased serum receptor activator of nuclear factor κB ligand (RANKL), a required factor for osteoclast formation, and increased osteoprotegerin (OPG), an inhibitor of RANKL, compared with OVX alone. Again, it is not clear if the effects of aspirin are specific for OVX-induced changes in osteoclasts or serum RANKL and OPG because sham-operated mice were not also given aspirin. Osteoclast formation can be stimulated *in vitro* by treating osteoclastic precursors, isolated from bone marrow or spleens, with macrophage-colony stimulating factor (M-CSF) and RANKL or by stimulating production of these factors with hormones or cytokines in osteoblastic cells. 1,25-OH vitamin D stimulated osteoclastic cell formation in co-cultures of primary osteoblasts with either bone marrow cells or spleen cells, which was inhibited by aspirin treatment. Aspirin treatment also decreased osteoclastic cell formation in spleen cells treated with M-CSF and RANKL. These results suggest that aspirin may decrease osteoclastogenesis both by regulating osteoblastic support of osteoclast formation and by inhibiting osteoclast formation directly.

This work suggests the potential of aspirin as an anti-osteoporosis drug, but questions remain. While the authors did show possible effects of aspirin on cell survival and BMMSC differentiation and telomere length, the direct mechanism of aspirin action was not identified. One of the major effects of aspirin is to inhibit prostaglandin (PG) production. Aspirin is an acetylated salicylate and acetylates a critical serine in the arachidonic acid binding site of cyclooxygenase (COX) 1 and 2, inhibiting the synthesis of PGs (5;6). Unlike other COX inhibitors (non-steroidal anti-inflammatory drugs, NSAIDs), this reaction produces irreversible inhibition of the enzymes. The role of PGs on bone maintenance is complicated, having effects on both bone formation and resorption (7). The *in vitro* effects of aspirin to inhibit osteoclast formation are similar to those seen with deficiency or inhibition of COX-2 (8). However, the *in vitro* effects of aspirin to stimulate osteoblastic mineralization are the opposite of the effects of COX-2 deficiency or inhibition (9). Exogenous PGE_2 increases both bone formation and bone resorption *in vivo* (10-15). Whether there is a net gain or

loss of bone appears to depend on the experimental model (16;17). Genetic deletion of COX-2, which is the predominant source of PGs in bone, results in a complex phenotype, including delayed fracture healing and a high bone turnover state associated with high serum parathyroid hormone (PTH) (18;19). Preliminary results from our laboratory have suggested that there is a greater loss in bone mass following OVX of COX-2 knockout mice, compared with wild type mice, however, these results may be confounded by high serum PTH in the knockout mice (20). Thus, if aspirin is increasing osteogenesis and decreasing resorption, these effects are likely to be not simply due to the inhibition of COX.

Aspirin and other salicylates have been described as having diverse actions, independent of effects on cyclooxygenase (21;22). These actions of salicylates may limit bone loss directly by acting on bone cells or indirectly by limiting inflammation or oxidative stress. Salicylates may inhibit nuclear factor κB, which is required for osteoclast formation stimulated by RANKL (23;24). Inhibition of nuclear factor κB by aspirin could contribute to the decreased osteoclast cells observed following aspirin treatment. Aspirin may also act by inhibiting cell adhesion and reducing oxidative stress (25-27). On the other hand, inhibitory actions of salicylates on the function of inflammatory cells could also prevent bone loss (21;22). Activated lymphocytes secrete pro-inflammatory cytokines that decrease osteoblastic function, while enhancing osteoclast formation, resulting in decreased bone mass (28). Thus, it is possible that the pleiotropic actions of aspirin may increase formation and decrease resorption of bone independently of the effects of aspirin to inhibit PGs. Use of non-acetylating salicylates and COX-deficient mouse models will help to clarify the mechanism of aspirin in limiting bone loss associated with estrogen deficiency.

Human epidemiologic studies suggest an association of aspirin with increased bone mineral density (BMD), although this is not consistently observed (29-32). A recent cross-sectional study of community-dwelling men and women older than 65 years assessed the association of aspirin and COX-2 selective inhibitors on BMD (32). In this study, men taking COX-2 inhibitors had lower BMD compared with men not taking any NSAID. Men using aspirin in addition to COX-2 inhibitors had a further reduction of BMD, compared with COX-2 inhibitor use alone. On the other hand, COX-2 inhibitors were associated with higher BMD in women not taking estrogen. Aspirin and COX-2 inhibitor use was associated with higher BMD than COX-2 inhibitor use alone in these women. Since low dose aspirin is widely used and relatively safe, prospective studies of its effects on the rapid bone loss that occurs at menopause, or even earlier during perimenopause, would be feasible and of great clinical interest.

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