Is aspirin treatment an appropriate intervention for osteoporosis?



"Pharmacologic targeted regulation of BMMSCs by aspirin may offer a new approach for estrogen-deficient osteoporosis treatment."

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Editorial
Future Rheumatology

Osteoporosis, the most prevalent skeletal disorder, is recognized by low bone mineral density (BMD) and structural deterioration of bone tissue, both of which lead to bone fragility fractures [1]. Postmenopausal osteoporosis is the most common and significant form of this disease, whereby the loss of estrogen causes an imbalance in bone metabolism. This imbalance is due to an overactivated osteoclast activity, and a temporal increase in osteoblast activity that is unable to rescue osteoclast-mediated bone resorption [1]. Although many systemic and local regulators are involved in estrogen-deficient osteoporosis, it appears that activated T lymphocytes are the key factor inducing osteoclast overactivation in postmenopausal osteoporosis [2–4]. Investigations focused on understanding the role of osteogenic cells in postmenopausal osteoporosis have been on the rise for several years. These studies demonstrate a potential link between cell death and osteoporosis [5,6]. It has been proposed that irregular apoptosis of osteoblasts/osteocytes leads to the imbalanced bone remodeling in osteoporosis [7,8]. To expand the knowledge of this form of osteoporosis so that the disease can be treated correctly, each aspect of the bone resorption and formation must be well understood. Currently, the role of osteoblasts and their progenitor bone marrow mesenchymal stem cells (BMMSCs) in osteoporosis is not well known.

BMMSCs are known as multipotent stem cells and are capable of differentiating into a variety of cell types including osteoblasts, chondrocytes, adipocytes and myoblasts [9–11]. The BMMSC/osteoblast lineage not only participates in *de novo* bone matrix formation to balance osteoclast-mediated bone resorption during the bone remodeling process, but also plays a critical role in maintaining homeostasis of the bone/marrow system [12]. This homeostasis includes governing the hematopoietic stem cell (HSC) niche [12–15] and modulation of immune cells, such as T and B lymphocytes, dendritic cells and natural killer (NK) cells [16–22]. Recently, transplantation of culture-expanded BMMSCs has been successfully used to treat a variety of clinical disorders, such as graft-versus-host-disease, via inhibiting T-lymphocyte proliferation and activity [23–25] and ameliorating HSC engraftment [26,27]. Since BMMSCs reside in the same marrow compartment with immune cells, it will be interesting to examine whether immune cells affect BMMSCs.

The deficiency of the Fas/Fas ligand system can cause various immune disorders associated with inappropriate T-lymphocyte proliferation, such as organ transplantation graft rejection, systemic lupus erythematosus and lymphoid tumors [28]. Expression of Fas/FasL on the lymphoid/myeloid lineage cells plays an important role in immune homeostasis, T lymphocytes and NK cell-mediated toxicity, as well as Fas-mediated tumor killing [29]. The current study showed that BMMSCs expressing Fas and CD3-activated T lymphocytes were capable of inducing BMMSC apoptosis in a direct cell co-culture system, but not in an indirect cell co-culture system [30]. By contrast, the perforin pathway, one of the major apoptotic mechanisms by T lymphocytes [31], was not involved in CD3-activated T-cell mediated BMMSC apoptosis. Furthermore, it was found that activated T lymphocytes failed to induce apoptosis in Fas-mutated BMMSCs. Therefore, the study suggested that the Fas/FasL pathway is a predominant cell death pathway in T-cell mediated BMMSC apoptosis [32].

In an effort to treat this form of osteoporosis, scientists have begun examining the use of a T-lymphocyte adoptive transfer system.

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Currently, the most often studied T-lymphocyte adoptive transfer system is used to study inflammatory bowel disease (IBD) [33,34]. Studies have shown that with an application of a widely used T-lymphocyte adoptive transfer system to immune-deficient recipient mice, $CD4^{+}CD45RB^{+/high}$ T lymphocytes account for the development of IBD. By contrast, transfer of the reciprocal $CD4^{+}CD45RB^{-/low}$ population not only failed to induce colitis, but also prevented the symptoms [35]. Interestingly, IBD patients ordinarily express decreased bone mass, an increased risk of developing osteoporosis and associated fragility fractures and morbidity [36,37]. However, the role of activated T lymphocytes on osteogenic progenitor cells in IBD patients has remained unclear. Adoptive transfer of $CD4^{+}CD45RB^{+/high}$ T lymphocytes into T-lymphocyte deficient mice with ovaryectomy, which lacked the osteoporosis phenotype due to the absence of T lymphocytes [38], demonstrated a typical BMD reduction and trabecular bone resorption in femurs [30]. Also, the impairment of BMMSCs was elucidated by several assays including colony-forming units fibroblastic (CFU-F) number, proliferation capacity and osteogenic capacity *in vitro* and *in vivo* [30]. As expected, osteoclast activity was upregulated in these $CD4^{+}CD45RB^{+/high}$ T-lymphocyte transfer mice by *in vivo* osteoclast assays, including an osteoclast-specific enzyme tartrate resistant acid phosphatase (TRAP) staining and in serum levels of soluble RANKL (sRANKL), a critical osteoclast differentiation factor. Furthermore, this upregulation was also seen in C-terminal telopeptide of type I collagen (CTX), a functional marker for osteoclast resorption. These findings provide direct evidence to support the hypothesis that interplays between T lymphocytes and BMMSCs may be critical for pathogenesis of osteoporosis.

While studies have investigated the role of T lymphocytes and osteoporosis, treatment measures have been researched to determine an appropriate pathway for these patients. Aspirin is a hugely popular and widely used NSAID. This drug is also known to prevent heart attacks by daily low-dose intervention. The effect of aspirin is shown in multiple biological pathways, such as inhibiting cyclooxygenase 2 (COX2) and cyclooxygenase 1 (COX1), and prostaglandin E2 activities. According to epidemiological studies, the regular use of aspirin or NSAIDs may have a moderate beneficial effect on BMD in postmenopausal women [39]; however, there appears to be no clinical significance regarding the protective effect on the subsequent risk of fractures [40]. Therefore, more detailed studies are necessary to examine whether aspirin is able to offer therapeutic effects to patients suffering from osteoporosis and, more importantly, to elucidate the mechanism by which aspirin may affect bone integrity.

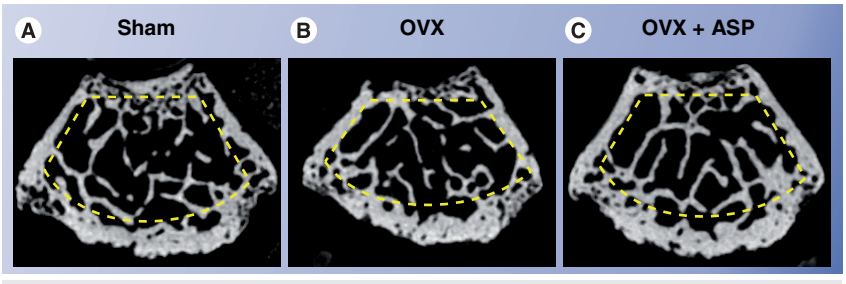

Figure 1. Representative horizontal µCT images of femurs. In OVX mice **(B)**, the femur showed the decrement of trabecular bone mass (area indicated by dotted line) when compared with the nonsurgery group **(A)**. Aspirin treatment (OVX + ASP; **(C)**) improved the bone mass in OVX mice.
ASP: Aspirin; OVX: Ovariectomized.

> "According to epidemiological studies, the regular use of aspirin or NSAIDs may have a moderate beneficial effect on BMD in postmenopausal women."

Women lose bone at a high rate during the initial years following menopause. Therefore, ovary-removed (ovariectomized [OVX]) mice are a suitable model to study osteoporosis. The estrogen-deficient mice show typical osteoporosis hallmarks such as reduction of BMD, reduced trabecular bone mass associated with overactivated osteoclast function (excess bone resorption) [41] and activation of T lymphocytes linked to osteoblast/osteocyte cell death [38]. Interestingly, estrogen-deficient OVX mice showed significant BMMSC damages including an increase in CFU-F number and cell proliferation, and a decrease in osteogenic capacity *in vitro* and *in vivo* [30]. When aspirin (0.6 mg/ml) was continuously given to OVX mice, their femurs showed a higher level of BMD than the control OXV, following 4 weeks of treatment (Figure 1) [30]. Aspirin was also shown to rescue impaired BMMSC function, such as recovering CFU-F number and osteogenic capacities. In addition, aspirin lessened osteoclast activity in OVX mice, as seen by decreased TRAP-positive cells and serum levels of sRANKL and CTX. When cultured BMMSCs were treated with aspirin, they showed improved anti-apoptotic capacity (Figure 2) and elevated mineralized tissue formation *in vitro* and *in vivo* [30]. Interestingly, aspirin was able to upregulate telomerase activity in BMMSCs *in vitro* [30], as seen in other cell types [42]. It was known that acquired telomerase activity in BMMSCs enhanced osteogenesis *in vitro* and *in vivo* via the Runx2 pathway [43]. Therefore,

upregulation of telomerase activity in BMMSCs may contribute to aspirin-mediated improvement of osteogenesis. Aspirin-elevated telomerase levels in BMMSCs were much lower than those in cancer cells, implying a safe use of aspirin to improve BMMSC functions.

Here, we provided experimental evidence that activated T lymphocytes are responsible for the BMMSC apoptosis through the Fas/FasL pathway, resulting in an accelerated osteoporosis phenotype in OVX mice. Moreover, aspirin appears to prevent osteoporosis by inhibiting BMMSC apoptosis and osteoclast-mediated bone resorption. Therefore, pharmacologic targeted regulation of BMMSCs by aspirin may offer a new approach for estrogen-deficient osteoporosis treatment. However, more detailed studies on the mechanism of aspirin-mediated anti-osteoporosis and proper dosing is critical to elucidate the role of aspirin in osteoporosis treatment.

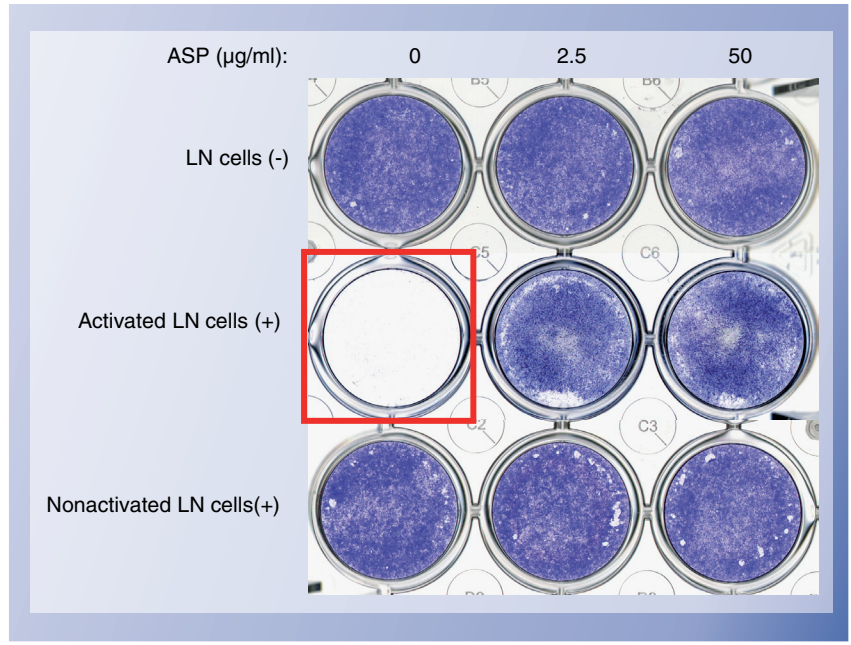

Figure 2. Representative images of co-culture with BMMSCs and LN cells.
BMMSCs were seeded on the culture wells, followed by co-culture with [LN$^+$] or without [LN$^-$] LN cells in the presence or absence of ASP at indicated concentrations. LN cells were either activated by plate-bound anti-CD3 antibody (1 μg/ml) for 3 days or not before the co-culture. 3 days after the co-culture, the wells were washed and stained with toluidine blue (indicated by the box). Activated LN cells induced BMMSC death as shown by the BMMSC-non-staining well, but ASP treatment rescues the BMMSC death under the co-culture with activated LN cells. Nonactivated LN cells were not capable of the BMMSC death stimulated with or without ASP.
ASP: Aspirin; BMMSC: Bone marrow mesenchymal stem cell; LN: Lymph node.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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