International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

A Study On Sequential Therapy Of PTH Therapy In Estrogen-Deficient Mice Model And Its Comparison With IL-17 Neutralizing Antibody

Dr.C.Suresh[1], Dr.M.Lakshmi[2], Dr.J.Sumitha[3], Dr.Ismail Gani[4], Dr.R.Ramkumar[5], Dr.N.Shankar[6], A.Anne Lino Jenina[7]

[1]Professor, Department of Electrical and Electronics Engineering, School of Engineering and Technology Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[2]Associate Professor, Department of Electrical and Electronics Engineering, School of Engineering and Technology, Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[3]Associate Professor, Department of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai,Tamilnadu,India.

[4]Assistant Professor, Department of Electrical and Electronics Engineering, School of Engineering and Technology, Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[5]Associate Professor, Department of Electrical and Electronics Engineering, School of Engineering and Technology, Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[6]Associate Professor, Department of Biomedical Engineering, School of Engineering and Technology ,Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[7]Assistant Professor, Department of Chemistry, School of Engineering and Technology ,Dhanalakshmi Srinivasan University, Samayapuram, Trichy,Tamilnadu, India

[1]sureshc.set@dsuniversity.ac.in , [2]lakshmim.set@dsuniversity.ac.in ,[3] jsumeetha@gmail.com

[4]ismailgani87@gmail.com , [5]2019ramkr@gmail.com ,[6] bmeshankar@gmail.com ,

[7]annelinojeninaa.set@dsuniversity.ac.in

Abstract

The global epidemic known as osteoporosis is characterized by the loss of systemic bone mass, poor bone strength brought on by low bone mineral density (BMD), and delayed bone mineralization, all of which raise the risk of non-traumatic fracture and bone fragility. Osteoporosis is the result of weakening or erosion of bone that causes the bone to become more porous. The word represents bone. Osteoporosis is mostly predicted by bone mineral density (BMD). Osteoporosis is defined as a BMD in young, healthy persons that is less than 2.5 standard deviations. According to recent research, inflammation is a major factor in the etiology of osteoporosis. T cell activation and bone loss are caused by low estrogen levels. According to a clinical study, pro-inflammatory cytokines are important factors that determine how quickly PMO women lose bone (Brincat, S. D., 2014). We can see from the literature that IL-17 cytokine is essential to the pathophysiology of osteoporosis. In this work, we have demonstrated that, in the bone defect/injury model, combination therapy (PTH (1-34) + IL-17 neutralizing antibody) increased bone regeneration potential relative to similar monotherapies. This suggested that a novel target for osteoporosis treatment drugs could be this combination therapy.

Key Words: *Sequential therapy, PTH therapy, estrogen-deficient, mice model, neutralizing antibody.*

INTRODUCTION

Antiresorptive and anabolic therapies are among the osteoporosis treatments available today and in the future. Teriparatide and abaloparatide are examples of anabolic treatments, whereas antiresorptive medications include bisphosphonate, SERM, calcitonin, HRT, etc. Anti-resorptive medications are generally associated with a number of negative side effects (Tu et al., 2018). Back pain, generalized musculoskeletal pain, high cholesterol, bladder inflammation, skin disorders, and mandibular osteonecrosis have all been associated with denosumab (Kuntze et al., 1989; Das and Crockett, 2013). The FDA has approved teriparatide (PTH 1-34), abaloparatide, and romosozumab as bone anabolic agents. One therapy option for osteoporosis that is part of the next generation is anti-sclerostin antibodies. Anti-sclerostin antibodies, cathepsin K inhibitors, SRC kinase

inhibitors, and other novel osteoporosis treatments (Voss et al., 2018). A monoclonal Romosozumab antibody was recently licensed by the FDA to treat osteoporosis in people who have a high risk of fractures (Markham, A. 2019). Severe cardiovascular events are a common side effect of romozumab (Gatti and Fassio, 2019).

Depending on whether PTH increases periodically or persistently, the hormone can have either catabolic or anabolic effects on the skeletal system (Silva BC, 2015). Teriparatide, the active, biological amino-terminal segment of PTH (1-34), is more suited to examine the osteoanabolic effects of PTH. PTH (1-34) treatment contributes to increased osteoblastogenesis, which suggests greater bone formation, via enhancing osteoblast survival. One of the possible mechanisms controlling PTH's anabolic effects in osteocytes is a reduction in SOST/sclerostin expression (Pacifici R. 2016). The "anabolic window," when PTH's capacity to make new bone appears to be at its peak, occurs in the early stages of PTH administration, when bone formation exceeds bone resorption. However, PTH's anabolic effects and the stimulation of its bone resorption arm gradually diminish over time. PTH (1-34) is therefore not recommended for longer than two years. As a result, after PTH is removed, an appropriate replacement is required. According to a number of small clinical trials, bone loss can be prevented after stopping PTH by substituting raloxifene, hormone replacement therapy, bisphosphonates (zoledronic acid, alendronate), or denosumab for PTH (1-34) or PTH (1-84) (Cosman, F. 2014; Black, D. M. et al., 2005; Rittmaster, R.S. et al., 2000). According to Altman et al., treatment with both PTH and the bisphosphonate alendronate (ALN) improved the trabecular structure at the rat proximal tibia more than when each treatment was administered alone (Altman, A. R. et al., 2014). Research by Omiya et al. revealed that, in comparison to monotherapy, successive treatment with neutralizing RANKL antibody after PTH discontinuation greatly increased BMD of cancellous and cortical bone volume (Omiya, T. et al. 2018). Following a two-year period, PTH combined with denosumab treatment resulted in enhanced cortical bone estimated strength and improved bone microarchitecture (Tsai, J. N., et al. 2016). Furthermore, denosumab and PTH combination therapy may be a significant treatment for primary osteoporotic individuals who are at high risk of vertebral fractures, according to Nakamura et al. (Nakamura, Y., et al. 2017). Unfortunately, the majority of these studies have brief follow-up periods and small sample sizes. Furthermore, even if the outcomes of these trials were encouraging, there is still room for discovery of fresh combinations because the long-term safety and effectiveness of these combinations are yet unknown.

Previous research conducted by our group shown that RANKL and TNF-alpha neutralizing Ab are not as effective as IL-17 neutralizing Ab (NIL17) in terms of immunoprotective and osteoprotective advantages (Tyagi, A. M., et al. 2014). Additionally, in comparison to monotherapy, we found that combination therapy (PTH+NIL17) exhibited synergistic skeletal benefits in Ovx mice (Mansoori, M. N., 2017). These findings motivated us to investigate NIL17's potential as a PTH replacement therapy. The determination of the ideal sequential treatment plan following PTH removal in postmenopausal osteoporotic women may result from this research.

MATERIAL AND METHODS

Ovx-induced osteopenic model and treatment procedure

The National Laboratory Animal Centre (NLAC) at the Central Drug Research Institute (CDRI), Lucknow, supplied all of the mice, which were then given a week to adjust to their new surroundings. Following that, the sixty mice were divided into six groups, each containing ten mice. Fifty mice were randomized to receive ovariectomy (Ovx) or sham surgeries performed with standard surgical methods (Dixit, M., et al. 2017). Ovx mice were left for four weeks following surgery to develop osteopenic state as a result of E2 deficiency. Based on their planned therapy, all of the mice in the Ovx group were randomly assigned to one of the five groups. The timetable depicted in figure 1 was followed for administering treatment therapy. The initial mouse groups used in the experiments were sham mice+vehicle (PBS); Ovx (ovariectomized) mice+vehicle (PBS); Ovx mice+PTH-Withdrawal (PTH-W) group; in this group, PTH 1-34 (40µg/kg/5day/week) was given for four weeks, then shifted to PBS for the remaining four weeks; Ovx mice+PTH-Continuous (continuous PTH therapy for 8 weeks); Ovx mice+ NIL17-Withdrawal (NIL17-W), twice a week, 100ng/mice neutralizing IL17 Ab was given for four weeks, then shifted to PBS for the remaining four weeks); Ovx mice+SHIFT, PTH 1-34 (40µg/kg/5day) was given for four weeks, then shifted to neutralizing IL17 Ab for the remaining four weeks.

Figure 1 describes the study and therapy timeline.

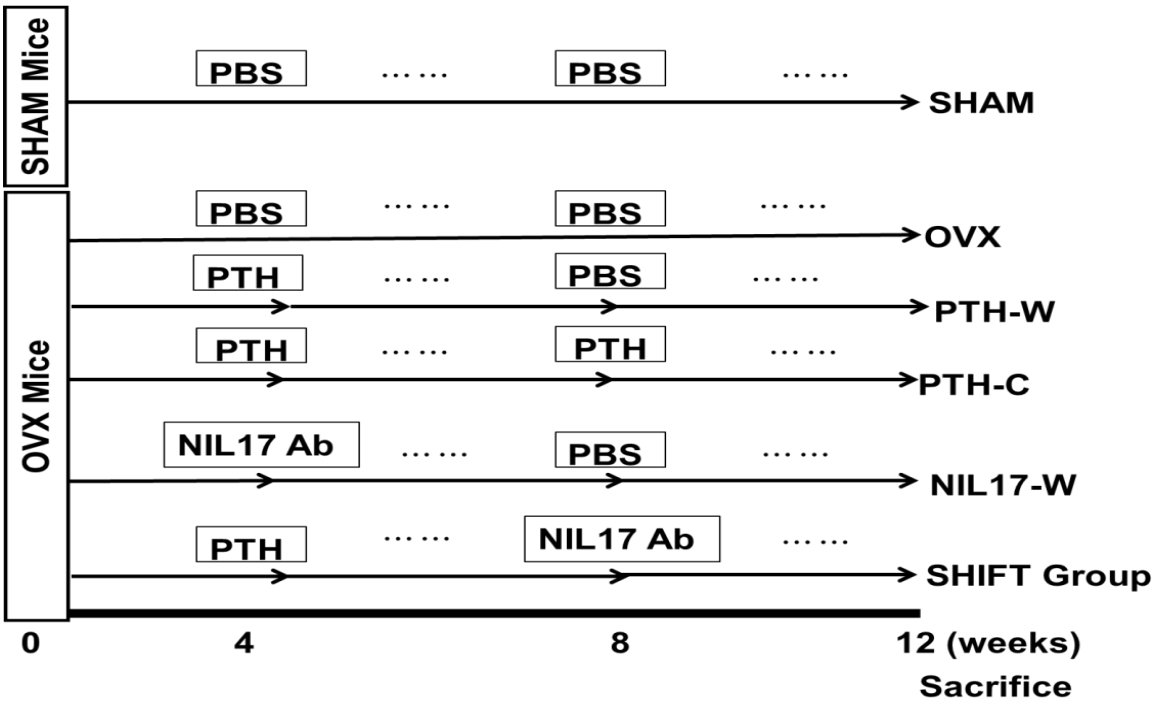


Figure 1. 12 weeks' study setup and overview of the timeline of therapy. Six groups of mice (N=10) were formed. Eight weeks following surgery, all animals were sacrificed.

Sample collection

During the 8 weeks of therapy duration, no adverse effects or deaths were detected. Eight weeks after therapy, all animals were euthanized. Both femur and tibia bones were collected from each mouse. Blood samples were taken for serum isolation via abdominal aorta puncture. Serum was collected by 4000 rpm centrifugation and kept at -80°C unless bone turnover biomarkers were examined. After removing any associated fatty tissue, the uteruses were taken out carefully, dry on tissue paper and weights were taken. Ovarian tissue disappearance and the appearance of significant uterine horn atrophy proved the success of the Ovx surgery during necropsy. Half of the bones were preserved at -80^{0}C for biomechanical testing and protein expression analysis and the rest half of the bones were incubated for 72 hours in paraformaldehyde, 4% (pH 7.0). These were then shifted for the remaining time in 70% isopropanol till micro CT scanning, BMD and histochemistry analysis.

In vitro studies:

Osteoblast culture

Calvaria of mice raised as pups were used to cultivate osteoblasts. To put it briefly, calvariae were removed from newborn pups (1-2 days old) of Balb/C mice, and five enzymatic breakdowns were subjected to 10 to 15 minutes of 0.1% collagenase and dispase. We used 10% α-MEM for the osteoblast cells. Cultures of cells were kept in 1% streptomycin/penicillin. Cells were maintained in a 37°C humidified incubator with 5% CO2 until 80–90% confluence was achieved. Trypsin was used to trypsinize the cells, and then they were reseeded in accordance with our experimental strategy (Kureel, J., et al., 2014).

Measurement of intracellular ROS level

2′, 7′-Dichlorofluorescin diacetate (DCFDA, Sigma Aldrich, USA) was employed to measure the ROS level in osteoblasts. In 12 well plates, osteoblast cells were planted at a density of 1×106 cells/well. For twenty-four hours, cells were cultured with 100ng/ml PTH and IL17 cytokine, both separately and together (Mansoori, M.N., et al., 2017; Tyagi, A.M., et al., 2012). Subsequently, the treated cells were exposed to 7 μM DCFDA for 30 minutes. Following that, cells underwent three PBS washes. Using

an Evos FL auto microscope, a fluorescent microscope, images were captured in order to determine the ROS level. Six fields of each condition were utilized to calculate the average fluorescence intensity during data analysis using Image J software (Zhang et al., 2018). Furthermore, the ROS level was ascertained using a fluorimeter-based methodology. Osteoblast cells were treated with PTH and IL-17 alone or in combination for a duration of 24 hours. Cells were treated with DCFDA (7 M) for 30 minutes at 37 oC prior to the conclusion of the experiment. Three PBS washes were performed on the cells. A fluorescence detector (SpectraMax M2) was used to measure the amount of fluorescence at 480 nm excitation and 535 nm emission (Trivedi, R., et al., 2020).

Statistical analysis

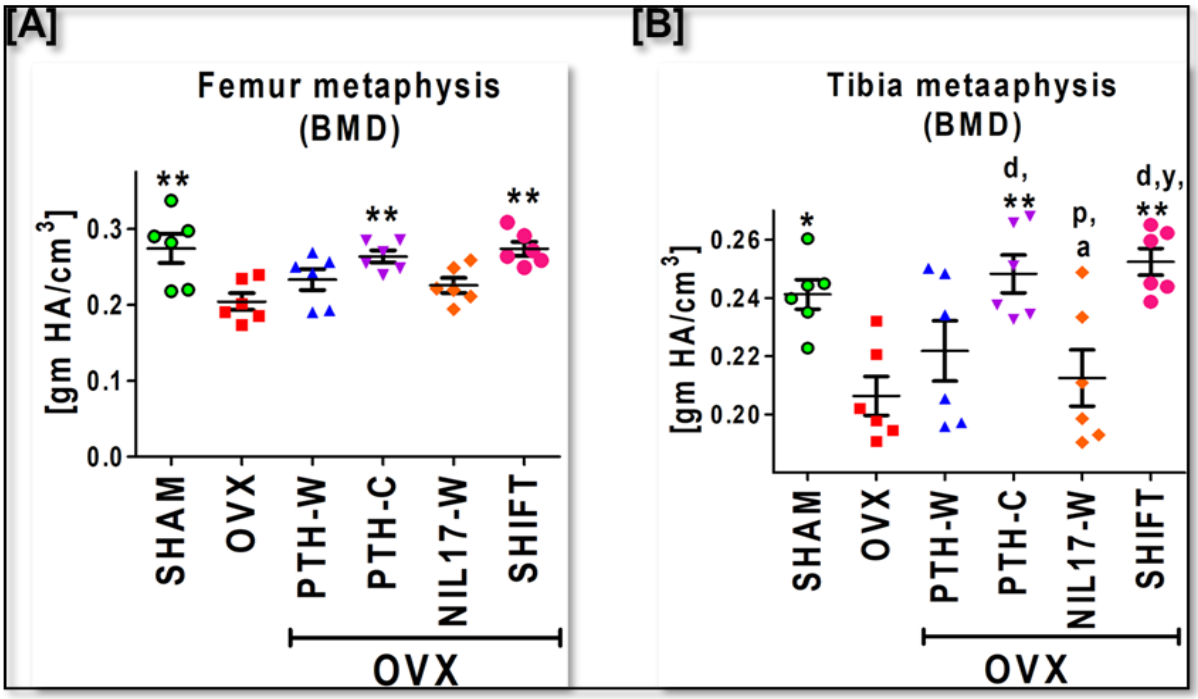
All the research data are expressed as mean±SEM. For research, utilizing Graph Pad prism 5, the data from different therapy set-up was evaluated to one-way ANOVA followed by a multiple significance comparison test by Newman Keuls. Significant P values were less than 0.05.

RESULTS

PTH to NIL17 shift therapy effect on BMD

Trabecular BMD lowered significantly in femur and tibia metaphysis bone in the Ovx group when compared with the sham group. A non-statistical and transitory rise in BMD was found in both the PTH and neutralization IL17 withdrawal groups. Continuous PTH [p>0.01] and SHIFT therapy [p>0.01] treatments regained BMD and were almost equivalent to the sham group in the metaphysis region of both femur and tibia **(Fig. 2A, 2B)**.

Figure 2 The effect of PTH (1-34) to neutralizing IL-17 shift on changes in BMD (A) Femur metaphysis BMD



(B) Tibia metaphysis BMD. (N=6/group).

SHIFT therapy's impact on trabecular parameters

A 3D-micro-CT net evaluation of the trabecular bones in the femur revealed that the Ovx animals had lost trabecular microarchitecture. Representative 3D scan pictures of the femoral trabecular microarchitecture bone from each group are displayed in Figure 3A. In comparison to the sham animals (Figs. 3E, F, G), ovx mice had higher Tb. Sp (trabecular bone separation), Tb. Pf (trabecular bone pattern factor), and SMI (structural model index) in their femur bones. They also had lower trabecular BV/TV (bone volume), Tb. Th (trabecular bone

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

thickness), Tb. No (trabecular number), and Connective density. Comparing the PTH-continuous group to the Ovx group, trabecular bone led to a significantly better trabecular BV/TV ratio ($p>0.001$) in the femur metaphysis. While there was a modest increase in BV/TV in the PTH-withdrawal group, it was still much lower than in the PTH-continuous group. In comparison to the Ovx group, the BV/TV % in the shift group was significantly higher ($p>0.001$). The PTH-continuous and SHIFT groups showed a significant improvement in Tb.Th and Tb.No.

In comparison to the Ovx group, the NIL17-withdrawal group displayed an increase in Tb. No (Fig. 3C and D). Figure 3E shows that the animals in the PTH-continuous ($p>0.001$) and shift groups ($p>0.001$) performed better in regaining the trabecular separation (Tb. Sp). When compared to the sham group, the Ovx group had significantly higher values for the structural model index (SMI) (Fig. 3G) and trabeculae pattern factor (Tb. Pf) (Fig. 3F). When compared to the sham group, the SHIFT ($p>0.01$) group had the best SMI preservation and pattern factor. The NIL17-withdrawal, PTH-continuous, and PTH-withdrawal groups did not affect the result. In comparison to the sham group, the PTH-continuous and shift groups exhibited a higher connection density (Fig. 3H).

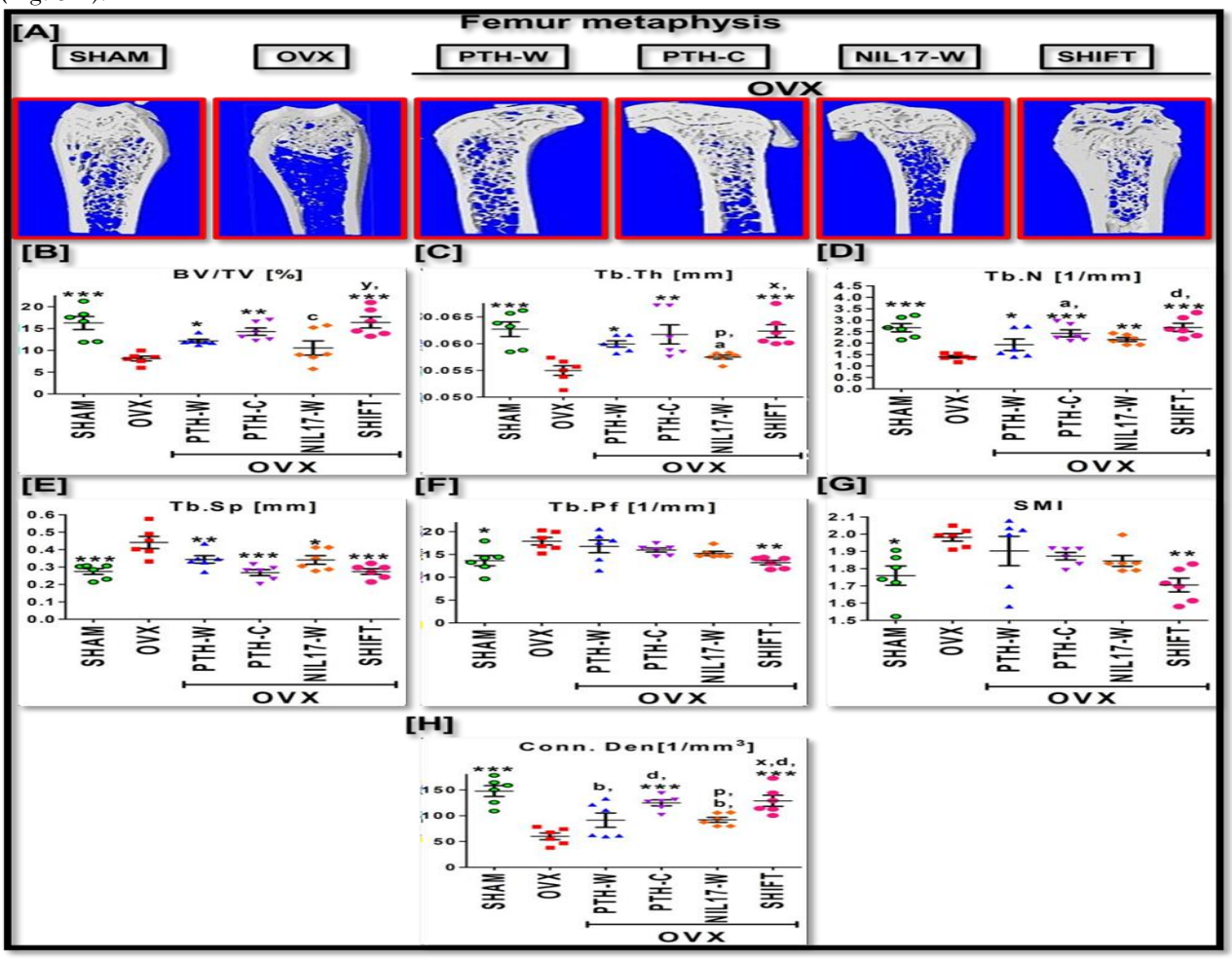


Figure 3. Effect of PTH to NIL17 shift therapy on the trabecular microarchitecture of the femur bone (A) Representative 3D images of femur trabecular microarchitecture bone. Microarchitecture parameters (B) trabecular BV/TV, (C) Tb.

Effect of SHIFT Therapy on biomechanical strength of bone

A 3-point bending biomechanical strength test was also done at the femur cortical region to determine the efficacy of shift therapy and other therapies on bone biomechanical strength. The Ovx group had lesser bone mechanical strength with significant power reductions ($p>0.01$), stiffness ($p>0.01$) and energy required to fail ($p>0.05$) than the sham group (Fig. 4A, B and C). We found out that the PTH-

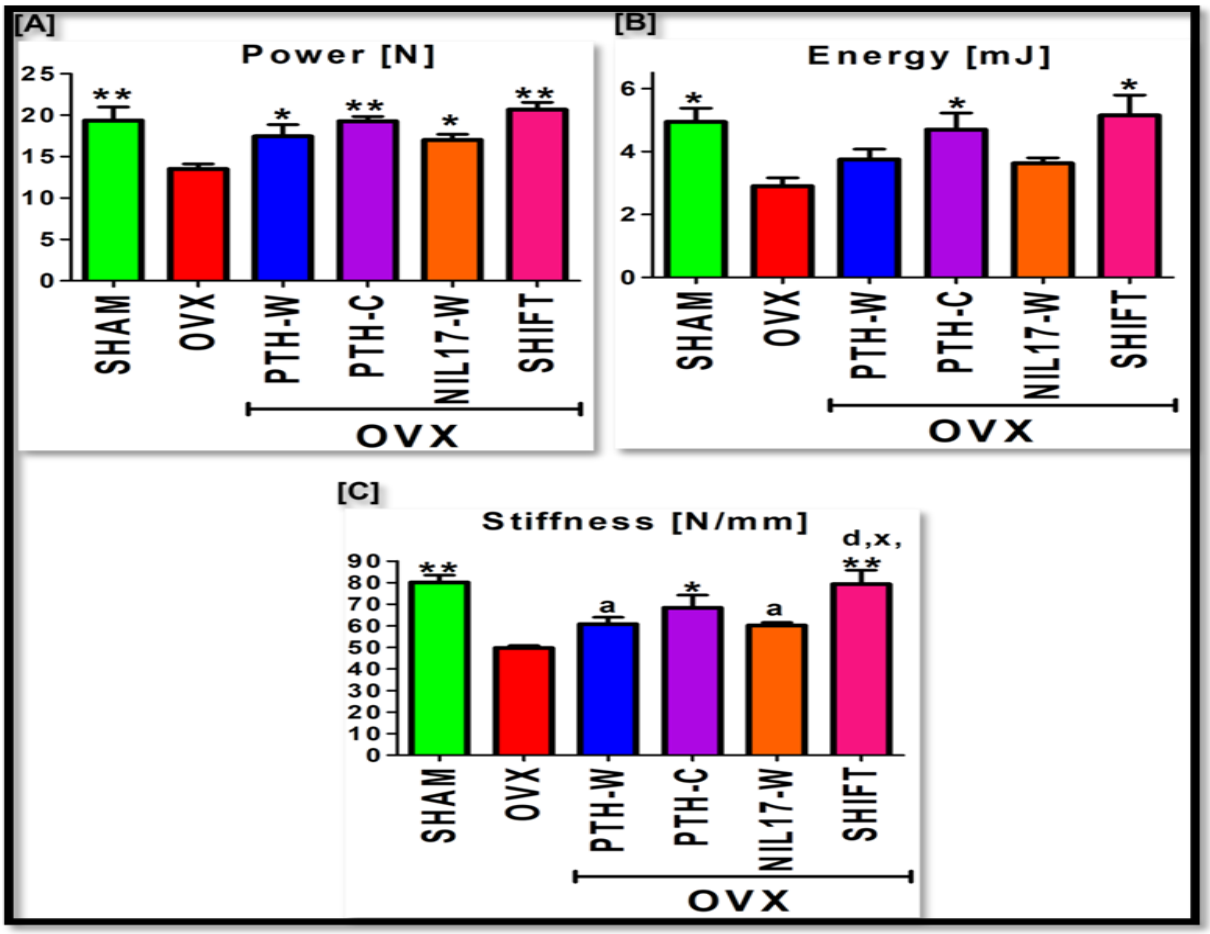
International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

withdrawal group and NIL17-withdrawal group showed significant improvements in power ($p>0.05$) but less in comparison with the PTH-continuous group and shift group ($p>0.01$). Increased stiffness was observed in the continuous PTH ($p>0.05$) and SHIFT ($p>0.01$) treatment group (Fig. 4A, B and C).

Figure 4. Effect of SHIFT treatment on femoral midshaft biomechanical strength parameters. Bone mechanical strength parameters were assessed in all groups. (A) Power (B) Energy and (C) Stiffness.

Effect of SHIFT Therapy on bone resorption markers

PTH primarily stimulates bone growth through bone lining cells on resting bone surfaces during the anabolic



window. Aslan D. et al. (2012) reported that although PTH therapy quickly increases the bone production marker, it also generates a parallel increase in the bone resorption marker. It is vital to examine the impact of several therapies, such as PTH withdrawal, PTH continuous, NIL17 withdrawal, and most significantly, SHIFT therapy, on osteoclastogenesis because long-term PTH therapy operated in a catabolic manner. The osteoclasts that were actively forming were identified using tartrate-resistant acid phosphatase (TRAP) staining. A sign of a functional osteoclast is TRAP. In bone sections from the Ovx, PTH-withdrawal, and PTH-continuous treatment groups, the number of trap+ve cells was significantly increased, but the NIL17-withdrawal group exhibited a significant decrease in TRAP-positive cells (Fig. 6A and B). Remarkably, PTH-stimulated rise in TRAP-positive cells was effectively reduced by sequential NIL17 Ab administration after PTH cessation (Fig. 5A and B).

A measure of bone resorption called CTX-I is correlated with both the overall number of osteoclasts and their ability to resorb bone in the blood. When comparing the Ovx mice (control) group to the sham animals, the level of blood CTX-I was higher ($p>0.001$). CTX-I levels were likewise higher in the PTH-withdrawal and PTH-continuous groups than they were in the NIL17-withdrawal and shift groups, where they were significantly

lower. Thus, shift therapy prevented the activation of the PTH-stimulated bone-resorption action (Fig. 5C).

Impact of SHIFT therapy on the formation of calcium nodules with mineralization

The production of mineralized calcium nodules in BMSC-differentiated osteoblast cells was also examined in relation to the effects of PTH-continuous and shift treatments. While there were more newly formed mineralized nodules in the PTH-withdrawal ($p>0.05$) and NIL17-withdrawal ($p>0.05$) groups compared to the Ovx group, they were not as numerous as in the shift therapy ($p>0.01$) and continuous PTH ($p>0.01$) groups (Figs. 5D and 5E).

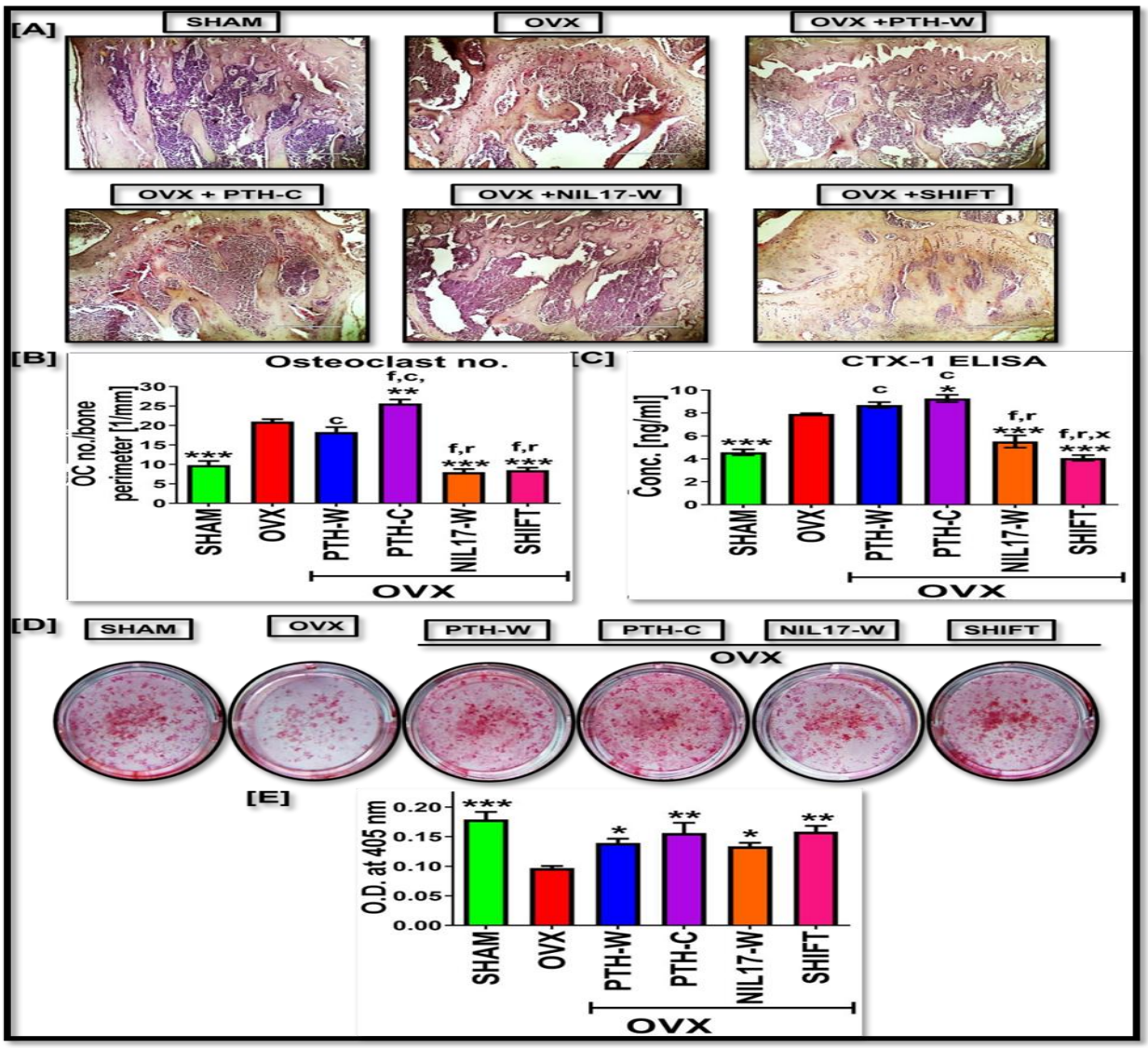


Figure 5. Effect of Shift therapy on functioning osteoclast and serum CTX-I level. Shift treatment induces the expression of mineralized nodules. (A) Representative images of functioning osteoclast in femur bone sections (5µm) from different therapy groups, (B) Osteoclast number (C) CTX-I Elisa (bone resorption marker), (D) Representative photograph of Alizarin-Red S stained calcium mineralized nodules (E) Alizarin stained calcium mineralized nodules were quantified.

SHIFT therapy's effect on oxidative stress marker

There is a considerable amount of oxidative stress generated at the site of bone loss. Our earlier research shown that the neutralizing IL17 antibody dramatically increases the expression of ATF4 and FOXO1 at the site of bone injury, hence reducing oxidative stress (Dixit, M., et al. 2017). Consequently, we also evaluated the

immunohistochemical expression of FOXO1 and ATF4. ATF4 and FOXO1 expression were significantly higher in the bone sections of the SHIFT group than in the other groups (Fig. 7A and B). Furthermore, ATF4 and FOXO1 expression was considerably increased in the SHIFT treatment group compared to the PTH-continuous group (Fig. 6A & B). Thus, shift therapy's higher bone-protective effect might be related to its anti-oxidative characteristics.

The expression of the ATF-4 protein was also investigated in bone tissue samples. ATF-4 protein levels were considerably higher in the sham, PTH-continuous, NIL17-withdrawal, and shift groups than in the Ovx (control) and PTH-withdrawal groups. It's interesting to note that the shift group's ATF-4 levels were greatest (Fig. 6C and D).

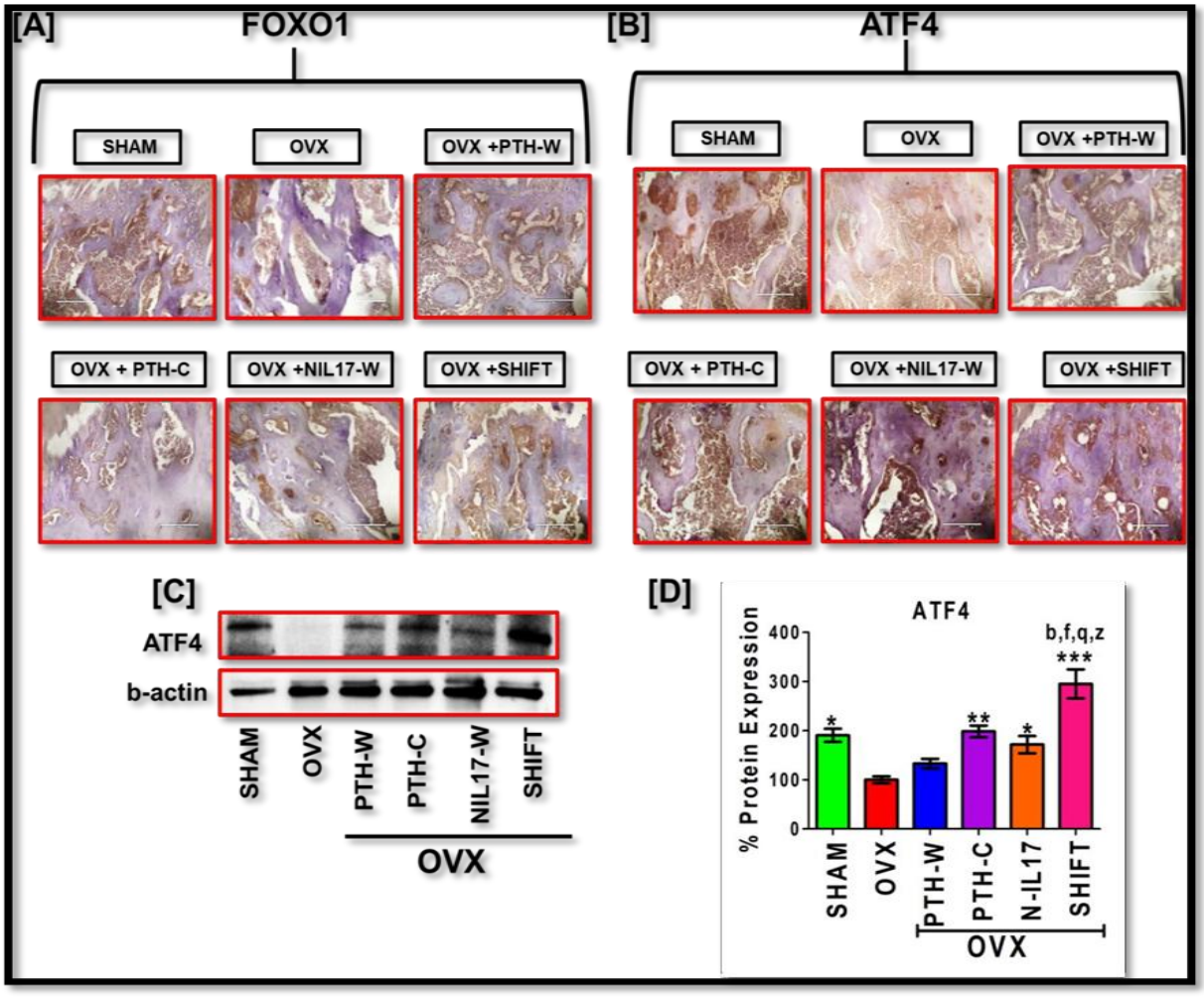


Figure 6. Immunohistochemical (IHC) localization and protein expression in the femoral trabecular bone region. (N=3). (A) FOXO1 (B) ATF4 expression in the imaged bone sections. Scale bars 200μm (C) ATF4 and FOXO1 protein expression levels (D) ATF4 densitometric analysis, (N=3).

PTH and the cytokine IL-17 effects on intracellular ROS level

Osteoblast cells' intracellular ROS level was assessed using EVOS FL Autofluorescence microscopy. PTH and IL-17 cytokine treatment were given to osteoblast cells in order to assess the impact of intracellular ROS (Fig. 7A). For a duration of 24 hours, osteoblast cells were treated with PTH and IL-17 cytokine, either alone or in

combination (PTH+IL 17 cytokine). The control group did not receive any therapy. Representative photos demonstrated that rising ROS levels were the cause of enhanced fluorescence (Fig. 7A). The photos were normalized to the backdrop using ImageJ software, which allowed for quantification. The results showed that ROS generation was significantly increased by cytokine IL-17 treatment in comparison to untreated (control) cells. Results from PTH therapy, either alone or in combination (PTH+IL17 cytokine), are not statistically significant. These results demonstrated that ROS (intracellular) generation is markedly increased by IL-17 (Fig. 7B).

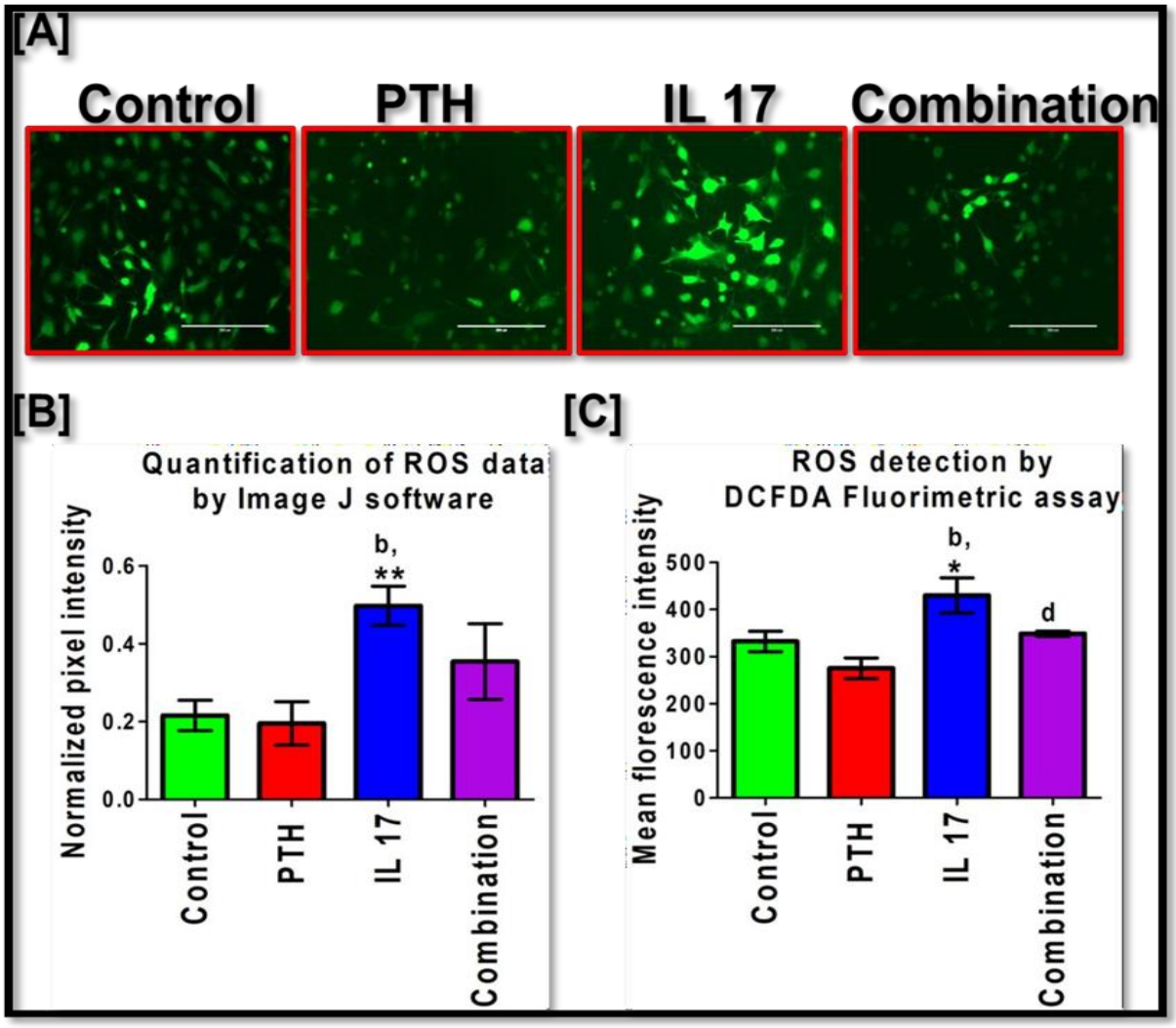


Figure 7. ROS level analysis (A) ROS levels in osteoblast cells were shown in fluorescence representative images. Scale bars, 200 μm (B) Quantification of fluorescence normalized ROS level in images by image J software. (C) DCFDA fluorimetric assay.

Using a fluorometric microplate assay, additional oxidative stress was measured by DCFDA oxidation in order to corroborate the ROS data mentioned above. When compared to untreated (control) cells, the IL-17 cytokine treatment group had a higher proportion of ROS because DCFDA was oxidized to brilliantly fluorescent 2',7' dichlorofluorescein (DCF). Results from PTH therapy alone or in conjunction with IL17 cytokine treatment (PTH+IL17) were not statistically significant (Fig. 7C). Therefore, shift therapy's improved effect on bone protection (PTH therapy moved to neutralizing IL 17) may be explained by its anti-oxidative qualities.

DISCUSSION

This study evaluated for the first time the effects of NIL17 on skeletal measures after PTH medication was stopped in an osteoporosis-affected Ovx mice model. While antiresorptive drugs were once the backbone of osteoporosis treatment, the advent of teriparatide or PTH gave patients another option for anabolic therapy that would build bone. Anabolic window development occurs during the early stages of PTH treatment because there is more bone growth than bone resorption. But after 24 months, PTH medication must be stopped because bone resorption progressively speeds up with the formation (Yeam, C. T. et al. 2018). Replacement therapy is necessary since the anabolic benefits of PTH are not sustained over an extended period of time. Anti-resorptive therapy can prevent the substantial decrease in bone mineral density (BMD) that occurs when postmenopausal women discontinue taking PTH (1-84) treatment (Black, D. M., et al. 2004). When PTH medication is switched to denosumab in individuals with postmenopausal osteoporosis (PMO), BMD gradually increases (Leder, B. Z., et al. 2015). Previous research suggests that the combination of denosumab and PTH increased BMD in postmenopausal women significantly more than the effects of either medication alone (Tsai, J. N., et al. 2013; Cosman, F., et al. 2008). Similar investigations using bisphosphonates in place of PTH have been conducted (Cosman, F., et al. 2008; Miller, P. D., et al. 2008; Boonen, S., et al. 2008; Cosman, F., et al. 2013; Tsai, J. N., et al. 2013). We made the decision to investigate whether consecutive treatment of NIL17 following PTH removal maintains the bone-anabolic effects of PTH, given the encouraging but inconsistent findings shown with sequential therapy of denosumab and bisphosphonates with PTH. Our study was based on earlier research conducted in our lab that demonstrated NIL17 had a greater immunoprotective and osteoprotective effect than TNF-α neutralizing antibody and RANKL (Tyagi, A. M., et al. 2014). Additionally, we demonstrated that the combined use of PTH and neutralizing IL17 antibody produced noticeably greater osteoprotective effects than either treatment alone (Mansoori, et al. 2017). The research was started using a model of osteopenic mice. In osteopenic mice, PTH was administered for four weeks, after which it was stopped and NIL17 was substituted for PTH for a further four weeks in order to assess the impact of PTH discontinuation on the bone network. We used a group receiving PTH treatment for eight weeks as a reference control. Bone mineral density and the trabecular and cortical bone network significantly deteriorated as a result of OVX. Even after PTH treatment was stopped, some trabecular markers increased relative to the Ovx group, but the SHIFT and PTH-C groups showed a stronger osteoprotective effect. The findings indicate that the anabolic benefits of PTH therapy diminish over time after discontinuation; however, this decline was averted by continuous NIL17 treatment. We speculate that the overall weakening of PTH's bone catabolic arm results from neutralizing IL17, an upstream cytokine that boosts PTH's bone resorptive action. The SHIFT therapy regimen demonstrated similar benefits to the 8-week PTH treatment and the sham group, which supported these data. Additionally, SHIFT and PTH-C therapy groups had the best presentations of cortical bone metrics. An established and recognized marker of osteogenic bone development is serum PINP (Hale, L. V., et al. 2007). The groups receiving PTH-C and SHIFT treatment had the highest serum PINP levels. Analyzing the bone biomechanical characteristics revealed a similar pattern. After PTH, sequential NIL17 treatment improved the energy, stiffness, and power characteristics of the bone. Conversely, PTH withdrawal caused a decline, indicating that PTH withdrawal therapy lowers the quality of bone.

PTH is a double-edged sword that, while it can promote bone formation, it also speeds up the resorption of existing bone. Increased osteoclast counts and serum CTX-I in withdrawal as well as 8-week PTH therapy groups demonstrated this. On the other hand, osteoclast counts and CTX-I levels were identical to those of the sham group in the SHIFT and NIL17 monotherapy group. Indeed, compared to the PTH-withdrawal group, the PTH-C group had a larger number of osteoclasts and serum CTX-I. Consequently, converting to NIL17 post-PTH therapy resulted in increased bone growth and decreased bone degradation at the same time. In order to create calcium nodules, osteoprogenitor cell recruitment was also encouraged by PTH-C and the shift group. This begged the question of how bone mass can be maintained at par with PTH-C and the sham group with NIL17 supplementation following PTH discontinuation.

An elevation in oxidative stress is caused by osteoporotic fractures or trauma injuries. Toxic metabolites such as reactive oxygen species (ROS) can harm proteins, lipids, and DNA, ultimately resulting in cell death. Cells counteract the negative effects of reactive oxygen species (ROS) by upregulating genes that repair DNA damage or enzymatic scavengers. This process includes dephosphorylating and activating a small family of widely distributed transcription factors called FOXOs (Liu, J.-W., et al. 2005; Lehtinen, M. K., et al. 2006). Redox equilibrium in osteoblasts depends on FOXO1, and oxidative damage is reduced by its interaction with the transcription factor ATF4. ATF4 also suppresses Th17 immunological responses, and FOXO1 directly opposes the RORt-Th17 program (Lainé, A., et al. 2015). (Xia, R., et al. 2015). Our earlier research has demonstrated that by encouraging the expression of FOXO1 and ATF4, anti-IL17 therapy reduced oxidative stress at the injury site (Dixit, M., et al. 2017). Furthermore, Yu et al. have shown that a deficit in ATF4 impairs the anabolic response by suppressing PTH-stimulated osteoblast proliferation and survival and eliminating PTH-induced osteoblast differentiation (Yu, S., et al. 2009). Consequently, we examined the expression of FOXO1 and ATF4 in each group's femoral regions. Compared to PTH-C and other groups, FOXO1 and ATF4 levels were shown to be more significantly increased by PTH-NIL-17 sequential treatment. Studies using western blotting confirmed these findings.

Furthermore, compared to the PTH and PTH+IL-17 groups, treatment of calvarial osteoblast cells with IL-17 increased the levels of ROS generation. This data implies that when PTH is administered in combination or as a sequential therapy, neutralization of IL-17 reduces ROS generation and therefore increases PTH's anti-oxidative activity. This may be the cause of the ability of IL-17 antibody therapy to maintain the bone-protective benefits of PTH after PTH removal. When combined, our results provide credence to the IL17 neutralizing antibody as a viable alternative treatment once PTH is stopped. But there are certain restrictions that have to be taken into consideration. Because laboratory animals were employed in our experiments, care must be taken when applying the research findings in clinical settings. It is necessary to examine regulatory toxicity and safety parameters. Second, it is still unclear how PTH and the NIL-17 antibody may affect a particular spot. Further basic science and clinical research will be required to comprehend the effects of SHIFT osteoporosis therapy on skeletal features.

CONCLUSIONS

In summary, the shift therapy group found that receiving neutralizing IL17 medication after PTH therapy was a significantly more effective course of action due to its ability to strengthen and increase the amount of bone that has been weakened due to PTH withdrawal. The reduction of total oxidative stress in the shift treatment group was demonstrated by the overexpression of factors including FOXO1 and ATF-4, which decrease stress created by ROS and improve osteoblast survival, ultimately leading to a rise in bone mineral density. The results of this trial offer solid support for the restoration of bone loss in osteoporotic patients following PTH cessation by the use of neutralizing IL-17A antibodies. Comparing PTH followed by NIL17 antibody therapy to other therapies like PTH followed by bisphosphonate or PTH followed by denosumab will be fascinating to observe. The results of this study may aid in the development of a more effective sequential therapeutic plan for the long-term management of osteoporosis.

REFERENCES

1. Tu, K. N., Lie, J. D., Wan, C., Cameron, M., Austel, A. G., Nguyen, J. K., Van, K., & Hyun, D. (2018). Osteoporosis: A Review of Treatment Options. P & T: a peer-reviewed journal for formulary management, 43(2), 92–104.
2. Kuntze, P. M., Fitzpatrick, S. C., Troendle, G., Hamilton, D. R., Vanderveen, J., Veiga, R. V., & Danello, M. A. (1989). Conference executive summary: priority education messages on the prevention and treatment of osteoporosis. Public health reports (Washington, D.C.: 1974), 104 Suppl(Suppl), 1–6.
3. Das, S., & Crockett, J. C. (2013). Osteoporosis - a current view of pharmacological prevention and treatment. Drug design, development and therapy, 7, 435–448. https://doi.org/10.2147/DDDT.S31504
4. Voss, P. J., Steybe, D., Poxleitner, P., Schmelzeisen, R., Munzenmayer, C., Fuellgraf, H., Stricker, A., & Semper-Hogg, W. (2018). Osteonecrosis of the jaw in patients transitioning from bisphosphonates to denosumab treatment for osteoporosis. Odontology, 106(4), 469–480. https://doi.org/10.1007/s10266-018-0362-5

5. Markham A. (2019). Romosozumab: First Global Approval. Drugs, 79(4), 471–476. https://doi.org/10.1007/s40265-019-01072-6
6. Silva, B. C., Costa, A. G., Cusano, N. E., Kousteni, S., & Bilezikian, J. P. (2011). Catabolic and anabolic actions of parathyroid hormone on the skeleton. Journal of endocrinological investigation, 34(10), 801–810. https://doi.org/10.3275/7925
7. Pacifici, R., Brown, C., Puscheck, E., Friedrich, E., Slatopolsky, E., Maggio, D., & Avioli, L. V. (1991). Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proceedings of the National Academy of Sciences, 88(12), 5134-5138.
8. Parfitt A. M. (2002). Parathyroid hormone and periosteal bone expansion. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 17(10), 1741–1743. https://doi.org/10.1359/jbmr.2002.17.10.1741
9. Cosman F. (2014). Anabolic and antiresorptive therapy for osteoporosis: combination and sequential approaches. Current osteoporosis reports, 12(4), 385–395. https://doi.org/10.1007/s11914-014-0237-9
10. Cosman, F., Nieves, J. W., & Dempster, D. W. (2017). Treatment Sequence Matters: Anabolic and Antiresorptive Therapy for Osteoporosis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 32(2), 198–202. https://doi.org/10.1002/jbmr.3051
11. Cosman, F., Nieves, J., Woelfert, L., Formica, C., Gordon, S., Shen, V., & Lindsay, R. (2001). Parathyroid hormone added to established hormone therapy: effects on vertebral fracture and maintenance of bone mass after parathyroid hormone withdrawal. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research, 16(5), 925–931. https://doi.org/10.1359/jbmr.2001.16.5.925.
12. Black, D. M., Bilezikian, J. P., Ensrud, K. E., Greenspan, S. L., Palermo, L., Hue, T., et al. (2005). One Year of Alendronate after One Year of Parathyroid Hormone (1–84) for Osteoporosis. New England Journal of Medicine 353, 555–565. Doi:10.1056/nejmoa050336.
13. Atkins, G. J., & Findlay, D. M. (2012). Osteocyte regulation of bone mineral: a little give and take. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 23(8), 2067–2079. https://doi.org/10.1007/s00198-012-1915-z
14. Rittmaster, R. S., Bolognese, M., Ettinger, M. P., Hanley, D. A., Hodsman, A. B., Kendler, D. L., & Rosen, C. J. (2000). Enhancement of bone mass in osteoporotic women with parathyroid hormone followed by alendronate. The Journal of clinical endocrinology and metabolism, 85(6), 2129–2134. https://doi.org/10.1210/jcem.85.6.6614
15. Altman, A. R., Tseng, W. J., de Bakker, C. M., Huh, B. K., Chandra, A., Qin, L., & Liu, X. S. (2014). A closer look at the immediate trabecula response to combined parathyroid hormone and alendronate treatment. Bone, 61, 149–157. https://doi.org/10.1016/j.bone.2014.01.008.
16. Omiya, T., & Tanaka, S. (2015). Nihon rinsho. Japanese journal of clinical medicine, 73(10), 1690–1695.
17. Omiya, T., Hirose, J., Hasegawa, T., Amizuka, N., Omata, Y., Izawa, N., et al. (2018). The effect of switching from teriparatide to anti-RANKL antibody on cancellous and cortical bone in ovariectomized mice.. Bone 107, 18–26. doi:10.1016/j.bone.2017.10.021.
18. Tsai, J. N., Uihlein, A. V., Burnett-Bowie, S. M., Neer, R. M., Derrico, N. P., Lee, H., Bouxsein, M. L., & Leder, B. Z. (2016). Effects of Two Years of Teriparatide, Denosumab, or Both on Bone Microarchitecture and Strength (DATA- HRpQCT study). The Journal of clinical endocrinology and metabolism, 101(5), 2023–2030. https://doi.org/10.1210/jc.2016-1160
19. Tsai, J. N., Uihlein, A. V., Lee, H., Kumbhani, R., Siwila-Sackman, E., McKay, E. A., Burnett-Bowie, S. A., Neer, R. M., & Leder, B. Z. (2013). Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: the DATA study randomised trial. Lancet (London, England), 382(9886), 50– 56. https://doi.org/10.1016/S0140-6736(13)60856-9
20. Kamiya, S., Nakamura, C., Fukawa, T., Ono, K., Ohwaki, T., Yoshimoto, T., & Wada, S. (2007). Effects of IL-23 and IL-27 on osteoblasts and osteoclasts: inhibitory effects on osteoclast differentiation. Journal of bone and mineral metabolism, 25(5), 277–285. https://doi.org/10.1007/s00774-007-0766-8
21. Kawaguchi, H., Oka, H., Jingushi, S., Izumi, T., Fukunaga, M., Sato, K., Matsushita, T., Nakamura, K., & TESK Group (2010). A local application of recombinant human fibroblast growth factor 2 for tibial shaft fractures: A randomized, placebo-controlled trial. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 25(12), 2735–2743. https://doi.org/10.1002/jbmr.146
22. Tyagi, A. M., Mansoori, M. N., Srivastava, K., Khan, M. P., Kureel, J., Dixit, M., & Singh, D. (2014). Enhanced immunoprotective effects by anti-IL-17 antibody translate to improved skeletal parameters under estrogen deficiency compared with anti-RANKL and anti-TNF-α antibodies. Journal of Bone and Mineral Research, 29(9), 1981-1992.
23. Tyagi, A. M., Srivastava, K., Mansoori, M. N., Trivedi, R., Chattopadhyay, N., & Singh, D. (2012). Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: a new candidate in the pathogenesis of osteoporosis. PloS one, 7(9), e44552. https://doi.org/10.1371/journal.pone.0044552
24. Mansoori, M. N., Shukla, P., & Singh, D. (2017). Combination of PTH (1-34) with anti-IL17 prevents bone loss by inhibiting IL-17/N-cadherin mediated disruption of PTHR1/LRP-6 interaction. Bone, 105, 226-236.
25. Singh, K. B., Dixit, M., Dev, K., Maurya, R., & Singh, D. (2017). Formononetin, a methoxy isoflavone, enhances bone regeneration in a mouse model of cor Pacifici tical bone defect. British Journal of Nutrition, 117(11), 1511-1522.
26. Dixit, M., Raghuvanshi, A., Gupta, C. P., Kureel, J., Mansoori, M. N., Shukla, P., John, A. A., Singh, K., Purohit, D., Awasthi, P., Singh, D., & Goel, A. (2015). Medicarpin, a Natural Pterocarpan, Heals Cortical Bone Defect by Activation of Notch and Wnt

Canonical Signaling Pathways. PloS one, 10(12), e0144541. https://doi.org/10.1371/journal.pone.0144541
27. Dixit, M., Singh, K. B., Prakash, R., & Singh, D. (2017). Functional block of IL-17 cytokine promotes bone healing by augmenting FOXO1 and ATF4 activity in cortical bone defect model. Osteoporosis International, 28(7), 2207-2220.
28. Kureel, J., Dixit, M., Tyagi, A. M., Mansoori, M. N., Srivastava, K., Raghuvanshi, A., Maurya, R., Trivedi, R., Goel, A., & Singh, D. (2014). miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation. Cell death & disease, 5(2), e1050. https://doi.org/10.1038/cddis.2014.4
29. Mansoori, M. N., Shukla, P., & Singh, D. (2017). Combination of PTH (1-34) with anti-IL17 prevents bone loss by inhibiting IL-17/N-cadherin mediated disruption of PTHR1/LRP-6 interaction. Bone, 105, 226-236.
30. Tyagi, A. M., Mansoori, M. N., Srivastava, K., Khan, M. P., Kureel, J., Dixit, M., & Singh, D. (2014). Enhanced immunoprotective effects by anti-IL-17 antibody translate to improved skeletal parameters under estrogen deficiency compared with anti-RANKL and anti-TNF-α antibodies. Journal of Bone and Mineral Research, 29(9), 1981-1992.
31. Tyagi, A. M., Srivastava, K., Mansoori, M. N., Trivedi, R., Chattopadhyay, N., & Singh, D. (2012). Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: a new candidate in the pathogenesis of osteoporosis. PloS one, 7(9), e44552. https://doi.org/10.1371/journal.pone.0044552
32. Zhang, S., Liu, Y., & Liang, Q. (2018). Low-dose dexamethasone affects osteoblast viability by inducing autophagy via intracellular ROS. Molecular medicine reports, 17(3), 4307–4316. https://doi.org/10.3892/mmr.2018.8461
33. Trivedi, R., Adhikary, S., Kothari, P., Ahmad, N., Mittapelly, N., Pandey, G., Mishra, P.R., Self-emulsifying formulation of Spinacia oleracea reduces the dose and escalates bioavailability of bioactive compounds to accelerate fracture repair in rats, Clin. Phytosci. 6 (1) (2020) 1–15.