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Review Article

Impact on Bone Health of the Drugs Administered to Mitigate High Altitude Illnesses: A Review

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ABSTRACT

The physiological mechanisms and organ systems of the body are affected at high altitude due to decreased oxygen availability, sometimes leading to different high-altitude disorders. The impact of high-altitude exposure on bone health is less conspicuous compared to other organs like the heart, lungs, brain, etc. Nonetheless, it is implied by *in vivo* studies on both animals and humans that there is a considerable reduction in bone mass and bone strength on exposure to high altitude. However, the probable repercussions of the use of high-altitude medication on bone is yet to be elucidated. The various drugs taken for prophylaxis, mitigation, and treatment of high-altitude conditions may have a considerable impact on Bone Mineral Density (BMD) and on the differentiation, proliferation, and activity of bone cells. This review focuses on the potential effect on bone health of various drugs taken for high altitude sickness, including acetazolamide, dexamethasone, aspirin, nifedipine, tadalafil, sildenafil, and ibuprofen.

Keywords: High altitude illness; Acetazolamide; Dexamethasone; Aspirin; High altitude medication; Bone health

INTRODUCTION

The skeletal system is a formidable part of the body with the functional unit of bone. It is resilient enough to go through vigorous physical activity, is able to rapidly adapt to various changes, and is flexible enough for intricate movement. The strength of the bone lies in its inherent features including size, structure, density, mass, mineral composition and shape. Various stresses are inflicted upon the bone due to genetic and lifestyle factors for which it often finds adaptations.

When traveling to or staying at high altitudes, a decrease in the partial pressure of oxygen is observed. This may impart another stress factor to bone tissues. It is implied by *in vivo* studies on both animals and humans that there is considerable reduction in bone mass and bone strength on exposure to high altitude. It has been reported that high altitude may also contribute to decrease in BMD. [1-3] The overall consequences of high-altitude exposure on the skeletal system and the possible underlying mechanisms behind bone loss induced by hypobaric hypoxia have been previously highlighted. [4] However, the probable repercussions of use of high-altitude medication on bone is yet to be elucidated.

Sometimes, the inability to acclimatize or rapid ascent leads to high-altitude disorders such as Acute Mountain Sickness (AMS), High-Altitude Cerebral Edema (HACE) and High-Altitude Pulmonary Edema (HAPE). They can even be life-threatening if not recognised early and common remedies include descent and supplementation of additional oxygen if needed. Some medications are also used in such cases, including acetazolamide, dexamethasone, aspirin, and nifedipine, and their mechanism of action are mentioned in Table 1. These medications are well-established as the prevalent treatment for high-altitude illnesses, and their influence on body functions is extensively studied. However, not much is known about the effect of these high-altitude medicines on bone health.

LITERATURE REVIEW

Acetazolamide

Acetazolamide is the most prevalent drug for prophylaxis of Acute Mountain Sickness (AMS) as it aids and speeds up acclimatization. A dosage of 250 mg twice a day for 3-5 days is recommended for both AMS and HACE [5].

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Its mode of action involves slowing down the hydration of carbon dioxide by inhibiting Carbonic Anhydrase (CA), leading to the accumulation of carbonic acid. The resulting acidosis stimulates the medullary respiratory centre which in turn increases ventilation and enhances oxygen delivery. Acetazolamide is a renal CA inhibitor which causes decrease in bicarbonate reabsorption and diuresis. It also represses production of Cerebrospinal Fluid (CSF), CSF pressure and inhibits antidiuretic hormone secretion [6].

Data suggests that Carbonic Anhydrase (CA) is implicated in calcification and supports bone formation and remodeling. Acetazolamide is a direct inhibitor of CA [7].

Carbonic Anhydrase II is an enzyme responsible for considerable generation of protons by osteoclasts. Acetazolamide is a specific inhibitor of CA II that contributes to low activity of bone resorption. Effect of acetazolamide on osteoclast and bone structure was studied *in vivo* and *in vitro*. The area of trabecular bone of the rats treated with acetazolamide was less than control group while there was a substantial decrease in the number of osteoclasts cultured with acetazolamide in comparison to the control group. There were morphological changes in some osteoclasts such as fragmentation of nuclei and shrinking of cytoplasm, suggesting apoptosis-like cell death. High doses of acetazolamide led to decreased bone resorption by inhibiting CA II and decreasing number of osteoclasts through cell death induction [8].

Parathyroid Hormone (PTH) induces various skeletal responses including changing the influx and efflux rates of phosphorus and calcium in the bone. When PTH was added to cultures, it led to increase in calcium and phosphate release, as anticipated. The effects of acetazolamide were also observed. Bones treated with both PTH and acetazolamide exhibited no rise in calcium release levels, as compared to bones treated with only acetazolamide [9].

The hypercalcemic response to Parathyroid Hormone (PTH) and to dibutyrul 3′,5′-cyclic AMP is inhibited by acetazolamide in the nephrectomized-parathyroidectomized rat. It has also been reported that in a rat model of disuse osteoporosis, adding acetazolamide to the diet can partially prevent denervation-induced bone loss [10,11].

Acetazolamide was also shown to affect acid production in isolated osteoclasts. There was a noteworthy reduction in intracellular acidity after treatment with acetazolamide, highlighting the significance of carbonic anhydrase for acid production in isolated osteoclasts [12].

Acetazolamide induces mild metabolic acidosis and inhibition of membrane-bound carbonic anhydrase in brush border of proximal tubules, leading to bone resorption *via* buffer effect of bone. This results in reduced Bone Mineral Density (BMD). Thus, acetazolamide is suggested as a potential treatment for diseases involving high BMD, such as sclerosing bone dysplasia and osteopetrosis. On treating patients with increased BMD with acetazolamide, satisfactory clinical response was observed with decrease in fracture and pain in osteopetrosis patients [13].

In post-menopausal white women who were chronic users of CA inhibitors, acetazolamide and methazolamide, for more than four years, bone-sparing effects could be seen on the spinal BMD [14].

When acetazolamide was administered along with exposure to hypobaric hypoxia in mice, it could not alleviate high altitude-induced bone loss and did not affect bone mineral content or BMD in comparison with only hypoxia-exposed mice. This could be because acetazolamide targets renal carbonic anhydrase and not osteoclastic carbonic anhydrase for inhibition. However, treatment with acetazolamide did not exhibit any detrimental effects on the skeleton and therefore, could be a favored substitute to dexamethasone for prophylaxis of AMS [15].

DISCUSSION

Acetazolamide also exhibits teratogenic effects in rats. Administration of acetazolamide to pregnant Wistar rats led to induction of limb abnormalities that were proportional to drug dosage [16].

Dexamethasone

Dexamethasone is a corticosteroid that is effectual in treating AMS symptom. However, the exact mode of action is not elucidated. Symptoms reappear after discontinuing dexamethasone, so it does not help in acclimatisation. It is preferred to administer it in conjunction with acetazolamide. It is also an exceptional drug for High Altitude Cerebral Edema (HACE), successful at higher doses. Administration of dexamethasone along with descent on onset of symptoms of HACE can be lifesaving [5].

The status of bone mineralization can be investigated through Bone Mineral Density (BMD), which is adversely affected as an effect of dexamethasone treatment. Dexamethasone has been identified as an independent risk factor for fracture and reduced BMD. It is extremely potent in suppressing growth when compared to other corticosteroids such as prednisolone. Its inhibitory effect on collagen synthesis was also higher when compared to the corticosteroid deflazacort, mentioned in Figure 1 [17,18].

In a study, dexamethasone suppressed bone formation and reduced bone turnover in SD rats, leading to decreased body weight, mineral apposition rate, bone matrix and bone formation rate. BMD was also reduced in some rats [19].

Bone Marrow-derived Stromal Cells (BMSCs) and Muscle tissue-derived Stromal Cells (MuSCs) are involved in ectopic bone formation, triggered by Bone Morphogenic Protein-2 (BMP-2). Dexamethasone augments cell proliferation in both BMSCs and MuSCs, leading to increased osteogenic capacity during osteogenic differentiation. In *in vivo* ectopic bone formation model, treatment with dexamethasone and BMP-2 doubly enhanced osteogenic capability compared to BMP-2 alone [20].

Synergistic use of dexamethasone and BMP-2 also elevated alkaline phosphatase levels in mouse pluripotent cells, which is considered a marker of osteoblast differentiation. Moreover, BMP-2 augments the dexamethasone/abscorbic acid/

glycerophosphate induced osteogenic differentiation in BMMSCs. They interact in several ways to modify bone formation [21].

Systemic influence of short-term dexamethasone treatment on bone metabolism was observed through induction of ectopic bone formation by BMP-7 injection in mice. Dexamethasone treatment led to higher osteoblast number and bone volume in ectopic bone nodules [22].

Particularly, antagonistic effects of dexamethasone on bone health have been observed in long-term glucocorticoid therapies. BMSCs exposed to dexamethasone are more prone to differentiate into adipocytes, potentially decreasing bone mass and increasing bone marrow fat. When Wnt/β-catenin pathway is activated, it mitigates the effect of dexamethasone on osteoblast/adipocyte balance, implying its involvement in dexamethasone-induced osteoporosis [23].

In BMSCs of young adult rats, dexamethasone increased alkaline phosphatase levels, osteoprogenitor differentiation and bone formation. Continued treatment with dexamethasone altered the stromal sub-population make-up and expression of haematopoietic lineage [24].

In treatment of chronic lung disease in preterm infants, dexamethasone suppressed collagen turnover in a dose-dependent manner with bone growth retardation [25].

Dexamethasone was shown to induce *Runx2* expression through activation of FHL2/ β-catenin-mediated transcription and enhanced activity of *Runx2* through TAZ (transcriptional co-activator with PDZ-binding motif) and MKP1 (Mitogen-Activated Protein Kinase (MAPK) phosphatase) upregulation during osteogenic differentiation in multipotent stem cells [26].

However, a recent study shows that dexamethasone induces osteogenic differentiation, not by directly upregulating *Runx2* as previously thought, but by inhibiting *SOX9* expression. In osteogenic cultures, upregulation of PPARG leads to development of adipocyte-like cells [27].

Dexamethasone has been known to trigger osteoblast apoptosis, increase alkaline phosphatase activity and inhibit cell proliferation. MC3T3-E1, an osteoblastic cell line, decreased in cell viability after exposure to dexamethasone. Paeoniflorin, the active compound of an herbal medicine, may recover the negative effects of dexamethasone on proliferation and promote differentiation of osteoblasts [28].

Expression of leptin and leptin receptor in human bone cells may increase on use of dexamethasone. Leptin has several influences on bone tissue regulation. Upregulation of leptin and leptin receptor by dexamethasone is related to downregulation of osteocalcin [29].

Treatment with dexamethasone may lead to an increase in activity of bone marrow erythroid cells. Dexamethasone indirectly induces erythropoiesis by triggering production of kidney erythropoietin [30].

The osteogenic cell sheet structure of bone marrow cells was improved when cultured with dexamethasone and ascorbic acid. Higher mRNA and protein expression of extra cellular matrix proteins was observed as well as increase in cell number and mechanical integrity, allowing the isolation of confluent cells in the cell sheet structure [31].

Dexamethasone significantly repressed basal manufacture of Interleukin *(IL)-6* and *IL-11* as well as PTH-stimulated *IL-1α*, or tumour necrosis factor-α in a dose-dependent manner. This implies that dexamethasone-induced bone loss is, at the very least, partly because of suppression of bone formation, including the inhibition of proliferation of osteoblast and formation of collagen [32].

In collagen-induced arthritic mice, dexamethasone potentiated bone erosion and osteoporosis by suppressing expression of *Il-6*, *IL-8* and *TNF-α* and triggering increased *RANKL* and *IL-17* expression *in vitro* and *in vivo* [33].

Dexamethasone interacts with various hormones and growth factors to influence bone formation and growth. Dexamethasone is shown to inhibit synthesis of Prostaglandin E2 in grain, q major hormone responsible for increase in bone mass and bone strength as well as involved in the bone healing process [34].

TGF-β and dexamethasone used in conjunction lead to stimulation of RANKL-induced osteoclastogenesis. Dexamethasone also reduced IGF-1 induced bone growth, *in vitro* in chondrocyte cell line and *in vivo* in chicken [35-37].

Sclereostin is an anabolic Wnt signaling pathway inhibitor with various glucocorticoid response elements. Dexamethasone is also known to alter the Wnt pathway. Sclereostin expression in osteoblasts is triggered on administration of dexamethasone. In isolated BMSCs, dexamethasone treatment suppressed formation of bone nodule but removal of sclereostin defended against this impediment in mineralisation. However, treatment with sclereostin antibody doesn't prevent the damaging effects of dexamethasone on bone growth [38,39].

Dexamethasone is also one of the most potent ligands inhibiting OPG production among glucocorticoids. It has shown 70%-80% inhibition of OPG in two human osteoblast cell lines. In neonatal mouse calvarial bones, dexamethasone induced bone resorption through regulation of the *RANKL*-RANK-OPG system and increased differentiation of osteoclasts. Dexamethasone and Vitamin D3 interaction augmented bone resorption [40,41].

Fibroblast Growth Factor-23 (FGF-23), secreted by osteoblasts and osteocytes, is a hormone that helps regulate reabsorption of renal phosphate and formation of calcitriol among other roles. Recently, it was found that dexamethasone inhibits *FGF23* secretion and increases expression of *DMP-1* and *Phex*, that encode *FGF-23* regulating genes. *FGF-23* has also shown cytoprotective effects against oxidative injury and cell death caused by dexamethasone in human osteoblast cells [42,43].

Aspirin

Aspirin is a Non-Steroidal Anti-Inflammatory Drug (NSAID) that acts as an inhibitor of cyclooxygenase and is used for its anti-coagulation, analgesic, antipyretic and blood thinning properties. It suppresses platelet activation by inhibiting the TXA2 pathway, prostaglandin-F2α and Cyclooxygenase-1 (COX-1), causing inhibition of platelet activation. Aspirin is often taken to prevent cardiovascular dysfunction and may cause side effects such as gastrointestinal tract bleeding, drug interaction, and resistance [44].

Headache is the principal symptom of Acute Mountain Sickness (AMS) and aspirin is commonly taken to relieve it. Aspirin prevents headache without improving oxygenation and exhibits less noticeable cardiorespiratory reactions to short-term exercise at high altitudes. During AMS, acute hypoxia accelerates prostaglandin concentration that causes activation of ergoreceptors and sympathetic stimulation. Aspirin diminishes these effects and alleviates the resulting headache [45].

Aspirin has been known to affect bone metabolism, as shown in Figure 1. Low doses of aspirin in users around 50-80 years of age was related to a higher BMD. Low doses of aspirin may also regulate bone cells, while high doses may be associated with independent excitation of osteoclast and osteoblast activity to decimate and create bone tissues [46,47].

Inhibitory effect of aspirin on osteoclast-like cells, through the NF-κB system was observed. Aspirin repressed differentiation of *RANKL*-induced osteoclasts and osteoclastogenesis by preventing activation of NF-κB and MAPKs in *RANKL*-induced monocyte/macrophage cell line [48].

Aspirin may also augment the survival of Bone Marrow Mesenchymal Stem Cells (BMMSCs) through prevention of Fas-induced apoptosis and enhancement of telomerase activity and telomerase length. Aspirin boosts expression of *Runx2*, ALP and osteocalcin; reduces serum *RANKL* and increases OPG levels. The deterioration of phospho-β-catenin and activation of Wnt signalling pathway is important for osteoblast formation [49].

TGF-β production in human BMMSCs is increased by aspirin which may lead to migration of MSCs to bone remodeling sites. Low doses aspirin might boost osteogenic ability of MSCs to alleviate bone loss from abnormal bone remodeling. On the other hand, aspirin at higher concentrations could exhibit an anti-proliferative effect on BMMSCs [50].

The bone protective effect of aspirin is also dependent on the COX-inhibitory activities. Aspirin and other COX-2 specific NSAIDs safeguard bone health. Aspirin could also be used as an adjuvant with Parathyroid Hormone (PTH). PTH increases both bone formation and resorption but the former to a higher degree. Aspirin can decrease bone resorption and augment the antiosteoporosis effects of PTH [51,52].

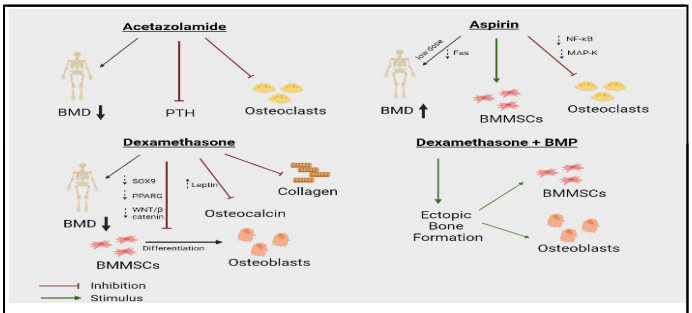

Figure 1: Effect of acetazolamide, aspirin and dexamethasone on bone health. **Note:** BMD: Bone Mineral Density; PTH: Parathyroid Hormone; BMMSCS: Bone Marrow Mesenchymal Stromal Cells; NF-κb: Nuclear Factor kappa B; MAPK: Mitogen-Activated Protein Kinase; PPARG: Peroxisome Proliferator-Activated Receptor Gamma

Nifedipine

Nifedipine is a calcium channel blocker that is used effectively for the treatment of HAPE. It works by decreasing pressure in the pulmonary artery and somewhat increasing arterial oxygenation. In people with a history of HAPE, prophylactic ingestion of nifedipine also prevents recurrence [53].

Nifedipine showed detrimental effects on epiphyseal plate thickness and bone turnover with significant morphological changed in the growth zone in a study on rabbits. Yet no adverse or beneficial consequences on bone turnover or bone and calcium metabolism were found in men who were chronic users of nifedipine. In BMMSCs and chondrocytes, treatment with nifedipine led to inhibition of mitochondrial respiration and rise in nitric oxide accumulation and pro-inflammatory activities, which may indicate a possible relation between cartilage health and the drug. Nifedipine has also been shown to significantly stimulate ALP activity in osteoblast [54-57].

Sildenafil

Sildenafil is a selective inhibitor of type-5 phosphodiesterase that lowers the pulmonary artery pressure and is a proposed treatment for HAPE. It suppresses HAPE by inducing vasodilation and promoting pulmonary gas exchange. It was hypothesized to assist in bone healing due to increasing blood supply to tissues and has shown contradictory effect on in rats, ameliorating the repair phase and suppressing the inflammatory phase of bone fracture healing. [58,59].

Tadalafil

Tadalafil is also an inhibitor of type-5 phosphodiesterase that has been successful in prophylaxis of HAPE for susceptible adults. It has been repurposed to increase bone mass in mice through anabolic and antiresorptive mechanisms. It has been observed to promote osteoblastic bone formation and hinder osteoclastic formation. It has also demonstrated accelerating effect on fracture healing in mice, similar to the effect of sildenafil [60-62].

Ibuprofen

Ibuprofen is a NSAID like aspirin that restricts production of prostaglandins and the inflammatory cascade through cyclooxygenase inhibition. It is efficacious in treating High Altitude Headache (HAH) and can be considered as an alternative of acetazolamide or dexamethasone for prevention of HAH. Ibuprofen suppressed differentiation and proliferation of bone marrow stromal cells into osteoblasts in a minipig model. Treatment with ibuprofen has also exhibited inhibition of osteoblastic proliferation *in vitro* with no additional effects on the cell cycle. Treatment with ibuprofen led to decreased bone strength in rabbits and inhibition of fracture healing in rats [63-67].

Table 1: High-altitude drugs and their mechanism of action.

Drug	Mechanism of action	Effect on bone
Acetazolamide	Carbonic anhydrase inhibitor, causes decrease in bicarbonate reabsorption, diuresis, and production of cerebrospinal fluid	May lead to decreased bone resorption, osteoclastogenesis and BMD
Dexamethasone	Corticosteroid, mode of action not elucidated	May decrease BMD, osteoblastogenesis, osteocalcin and collagen levels
Aspirin	Suppresses platelet activation by inhibiting the thromboxaneA2 pathway, prostaglandin-F2α and cyclooxygenase-1, causing inhibition of platelet activation	Low doses may increase BMD, Higher doses stimulate BMMSCS and inhibit osteoclasts
Nifedipine	Calcium channel blocker, decreases pressure in pulmonary artery and increases arterial oxygenation	May have detrimental effect on bone turnover
Tadalafil	Selective inhibitor of type-5 phosphodiesterase	Promote osteoblastic bone formation and hinder osteoclastic formation
Sildenafil	Selective inhibitor of type-5 phosphodiesterase	May suppress bone fracture healing
Ibuprofen	Restricts production of prostaglandins and the inflammatory cascade through cyclooxygenase inhibition	Suppressed osteoblastogenesis and bone healing

CONCLUSION

The various medication taken to prevent high altitude sickness can be seen to have mostly detrimental effects on bone health. The carbonic anhydrase inhibitor, acetazolamide decreased bone resorption and induced osteoclast cell death. It also had an inhibitory effect on PTH and led to an overall decrease in BMD. The corticosteroid, dexamethasone also reduced BMD and adversely affected osteoblastogenesis. It hampered production of collagen and osteocalcin. Synergistic use of dexamethasone and BMPs leads to ectopic bone formation. Aspirin is a NSAID that has exhibited increase in BMD in low doses as well as promoting survival of BMMSCs and boosting their osteogenic ability. However, it has an inhibitory effect on osteoclastogenesis. Other drugs administered at high altitudes, such as nifedipine, sildenafil, tadalafil, and ibuprofen, show varying effects on bone metabolism, but further research is needed to establish concrete results. Additionally, although all these drugs are used for the remediation of high-altitude sickness, their effect on bone has only been studied at sea level thus far. Further study on their impact on bone after administration at high-altitude is required.

CONFLICT OF INTEREST

Author(s) declare no conflict of interest.

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