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REVIEW

Organic nitrates for osteoporosis: an update

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The number of osteoporotic fractures is increasing worldwide as populations age. An inexpensive and widely available treatment is necessary to alleviate this increase in fractures. Current treatments decrease fractures at trabecular bone sites (spine) but have limited effects at cortical sites (hip, legs, forearm and upper arm)—the most common sites of osteoporotic fracture. Treatments are also limited by costs, side effects and lack of availability. Nitric oxide (NO) is a novel agent that has the potential to influence cortical bone, is inexpensive, widely available and has limited side effects. In this review, we will evaluate the in vitro and in vivo data that support the concept that NO is important in bone cell function, review the observational, case control and randomized trial data on organic nitrates and the effects of these agents on bone turnover, geometry and strength.

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Introduction

Osteoporosis is characterized by a reduction in bone mass and a disruption of skeletal microarchitecture, leading to an increased susceptibility to fracture with minimal trauma. Osteoporotic fractures are common, costly to treat, and cause pain, disability and premature death.[1] Approximately 30–50% of elderly women and 15–30% of elderly men will suffer a fracture related to osteoporosis in their lifetime.[2] As elderly men and women are the fastest growing group in the world and the incidence of osteoporotic fractures increases exponentially with age, the number of men and women with osteoporotic fractures is expected to increase dramatically over the next 50 years.[3]

Altered bone remodeling—excessive bone resorption and/or impaired bone formation—is a key risk factor for osteoporotic fracture.[4–6] For the most part, the bone remodeling cycle is tightly regulated such that bone formation is coupled with bone resorption and bone mass is maintained. The exceptions to this occur in childhood when bone formation exceeds resorption with a net bone gain[7] and in menopause and older age when resorption exceeds formation.[8] In addition to the independent effects of resorption on fracture risk, the increased resorption is associated with a decrease in bone mineral density (BMD) and there is a strong and consistent relationship between low BMD and an increased risk of fracture[9,10]

Given the importance of bone remodeling in fracture risk, it is not surprising that the majority of pharmacologic agents developed for the prevention and treatment of osteoporosis act by inhibiting bone resorption (hormone replacement therapy, bisphosphonates and selective estrogen receptor modulators) or by stimulating bone formation (parathyroid hormone). Agents that are currently available have some limitations; they only target one part of the bone remodeling cycle and because remodeling is coupled, the drugs will either increase or decrease both resorption and formation. There are limited long-term safety data (<10years), and generally speaking, these agents are costly and not available worldwide. An optimal agent would be one that decreased bone resorption while increasing bone formation to have maximum effects on BMD, is inexpensive and available worldwide. One potential agent is nitric oxide (NO), the subject of this review.

Nitric Oxide and Bone

***In vitro* and animal studies**. NO is a short-lived free radical involved in the regulation of many physiological processes, including bone remodeling.[11] NO is generated by the nitric oxide synthase enzymes (NOS) from molecular oxygen and the terminal guanidine nitrogen of the amino acid L-arginine, yielding L-citruilline as a coproduct;[12] NO can also be generated nonenzymatically from nitrite in the acid environment of the stomach and organic nitrates (for example, nitroglycerin (NTG), isosorbide mononitrate (ISMO) and isosorbide dinitrate) can act as NO donors.[12,13] The L-arginine-NO pathway is depicted in **Figure 1**.

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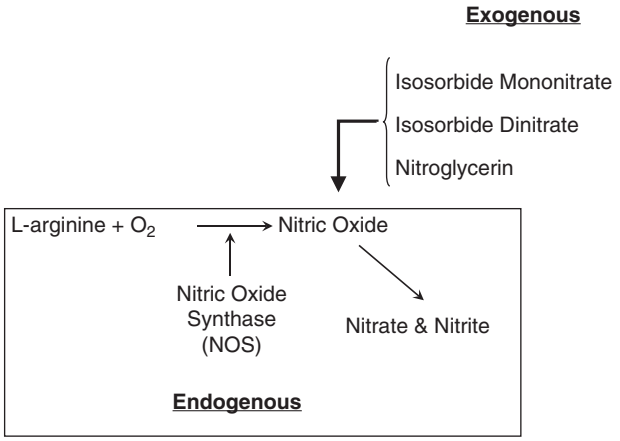

Figure 1 The L-arginine-nitric oxide (NO) pathway.[49] Endogenous NO is synthesized from L-arginine and molecular oxygen by the nitric oxide synthase (NOS) group of enzymes and reacts rapidly with oxygen to form nitrate (NO_3) and nitrite (NO_2). Exogenous sources of NO can be supplied via NO donors such as isosorbide mononitrate, isosorbide dinitrate and nitroglycerin.

Bone cells are able to generate NO. Specifically, osteoblasts produce endothelial nitric oxide synthase (eNOS) resulting in low levels of circulating NO whereas activated osteocytes produce high levels of NO via inducible nitric oxide synthase (iNOS). The NO generated by osteoblasts regulates osteoclast activity and acts as a signaling molecule in osteoblasts and osteocytes. *In vitro* studies demonstrate that NO has a biphasic effect on osteoclast activity and bone resorption;[14–18] low concentrations (as produced with activation of eNOS) potentiate bone resorption whereas high concentrations (as produced with activation of iNOS) inhibit activity.[19–21] The mechanism by which NO influences osteoclast activity may, in part occur via the receptor activator of NF-kappaB ligand (RANKL)/osteoprotegerin (OPG) pathway: high levels of NO stimulates OPG, OPG binds to RANKL that prevents the binding of RANKL to the receptor activator of NF-kappaB (RANK) and decreases osteoclast activity.[22]

The effects of NO on osteoblasts are less well-characterized. Some, but not all, studies report that low concentrations of NO stimulate osteoblast growth and differentiation.[23] Further, mice lacking NO synthase have defective bone formation due to defects in osteoblast differentiation and functioning, indicating that NO has a key role in regulating bone formation.[24,25]

Human studies. Plasma NO is reported to be positively correlated with estradiol levels and has been shown to increase with exogenous administration in humans.[26–28]

Our group first examined the relationship between the use of nitrates and BMD in humans using data from the Study of Osteoporotic Fractures (SOF), a multicentre, prospective, observational study of 9704 ambulatory, Caucasian women, aged 65 years and older.[29] We hypothesized that women taking nitrates intermittently would have significantly higher bone mass than those who took nitrates continuously. Continuous exposure to organic nitrate causes tachyphylaxis to its vascular effects. Data from the cardiovascular literature report tachyphylaxis to nitrates with increasing frequency of dose[30] Tachyphylaxis to nitrates may develop in bone: rats given NTG ointment daily for 12 weeks had increases in BMD similar to those with estrogen, yet more frequent administration abolished any beneficial effects.[31]

We compared hip and heel BMD among nitrate users (391 women) and nonusers (5827 women) identified by self-report. Women who reported using ISMO, isosorbide dinitrate, or NTG more than once a day, every day, were classified as continuous users ($n = 317$), and all other women were classified as intermittent users ($n = 74$). Compared with nonusers, nitrate users were more likely to have risk factors for low BMD.[32] After adjusting for these differences, and for estrogen use, we found that hip BMD was 2.6% higher and heel BMD was 5.3% higher among intermittent nitrate users compared with nonusers, and intermittent nitrate users had greater BMD than continuous users at both these sites. The results were consistent with our hypothesis that intermittent use of nitrates improves bone mass whereas continuous nitrate use may lead to tachyphylaxis.

There have also been two case control studies that have reported on the effect of organic nitrates on fracture risk.[33,34] Both of these studies report that nitrate use was associated with a decreased risk of fracture, including hip fracture (by about 10 to 15%), and that the reduction in fracture was greatest among those using low-dose nitrates on an as-needed basis. However, these studies were unable to compare formulations with regard to efficacy or headaches.

We conducted a randomized controlled trial comparing the effects of placebo and intermittent ISMO on markers of bone turnover in postmenopausal women. We randomly assigned 144 women ($\geqslant 3$ years postmenopausal with femoral neck BMD *T*-scores between 0 and -2.5) to 12 weeks of placebo or intermittent ISMO of 5 mg or 20 mg per day; typically ISMO is prescribed at 20 mg twice a day. We measured changes from baseline in urine N-telopeptide (NTx), a marker of bone resorption and serum bone-specific alkaline phosphatase (BSAP), a marker of bone formation.[35]

Our earlier work suggested that the effect of nitrates on bone was a class effect and as such we did not think the formulation nitrate we chose would result in substantially different effects on markers of bone turnover as long as it was given intermittently and in a low dose.[29] We chose to study ISMO because it is completely and consistently absorbed, does not have a first-pass effect, has linear dose-dependent pharmacokinetics and marked dose-dependent hemodynamic effects.[36] We chose doses of 5 mg and 20 mg; pharmacologic data demonstrate that the threshold of oral activity of ISMO is 5 mg and the maximum response is reached with doses of 20 mg.[30] To prevent tachyphylaxis,[37] we gave ISMO, which is typically administered twice a day, once a day or intermittently.

We found that, compared with placebo, women randomized to intermittent ISMO at 20 mg had a 45.4% decrease in NTx (95% confidence interval (CI): 25.8 to 64.9) and a 23.3% increase (95% CI: 8.9 to 37.8) in BSAP. Women randomized to intermittent ISMO at 5 mg had a 36.3% decrease in NTx (95% CI: 14.8 to 57.8) and a 15.9% increase in BSAP (95% CI: 1.1 to 30.7).[29]

The decreases in NTx that we observed with 20 mg of ISMO are similar to those reported with alendronate, risedronate, and estrogen (about 50%) and greater than the 25% decreases reported with raloxifene.[38,39] However, all of the antiresorptive agents concomitantly decrease rates of bone formation. In contrast, we observed that treatment with ISMO resulted in significant increases in BSAP. The decrease in resorption,

coupled with the increase in formation, suggests that ISMO may reduce fracture risk to an even greater degree than that seen with the current antiresorptive agents. The only adverse event was headache. Headaches, were more common among women randomized to ISMO (5 and 20 mg groups combined $n = 55$, 57%) compared with placebo ($n = 2$, 4%; $P = 0.004$). Headaches were no more common among women taking 20 mg of ISMO ($n = 28$) than among women taking 5 mg of ISMO ($n = 27$; $P = 0.7$).

A randomized trial compared the effects of transdermal NTG with estrogen for the prevention of oophorectomy-induced bone loss.[40] The 12 month study was an open labeled design: 16 women (age 36 to 45) received either conjugated estrogen (0.625 mg per day) ($n = 8$) or transdermal NTG (15 mg per day) ($n = 8$). After 6 months, women taking NTG ointment had a 40% decrease in NTx and 25% increase in BSAP compared with baseline. At 12 months, there was no difference, compared with baseline, in lumbar spine or total hip BMD, among women taking conjugated estrogen compared with those taking transdermal NTG. Headache incidence, which is commonly associated with nitrate use for cardiovascular disease, was not found to be an issue in this trial.

Considered together, these two trials suggest that the magnitude of effects on bone markers and the uncoupling between formation and resorption is similar for both NTG and ISMO. The lack of headaches with NTG deserved further study and this served as the basis for our next research project that consisted of a 4-week pilot study and a 27-month main study in healthy postmenopausal women. The aim of the pilot study was to determine the best-tolerated preparation of nitrate (NTG vs ISMO) for future studies. Specifically, we assigned 22 subjects to intermittent NTG at 15 mg per day and intermittent ISMO at 20 mg per day, each for 1 week. The order of the treatments was random, accompanied by a placebo control (identical in sight and smell to the active treatment). In between each treatment, there was a 2-week washout period. Subjects recorded the severity of headaches upon awakening every day for 4 weeks using a Visual Analog Scale (VAS). We calculated the mean headache score for each subject over both 7-day treatment periods and then the mean headache score, considering all subjects for each of the NTG and ISMO treatment periods. We found that the ISMO was associated with more frequent headaches (12 women reported daily headaches vs 5 women) and more severe headaches than the NTG ointment (mean headache score: 4/5 for ISMO vs 2/5 for NTG ointment). As a result, we used the NTG ointment for our next study (reviewed below) and published in the Journal of the American Medical Association.[41] To limit differential dropout due to headaches, the main trial followed from a 1-week nitrate run-in phase; women who discontinued the nitrate due to headaches did not enter the main trial.

Our trial was designed to test the effects of nitroglycerin on bone turnover, density, geometry and strength. We randomly assigned 243 postmenopausal women with BMD *T*-scores between 0 and -2.0 at the lumbar spine to 15 mg of NTG ointment or placebo daily at bedtime for 24 months.

At 2 years, compared with placebo, NTG increased spine BMD by 6.7% (95% confidence interval (CI)], 5.2 to 8.2; $P < 0.001$), femoral neck BMD 7.0% (95% CI, 5.5 to 8.5%; $P < 0.001$) and total hip BMD 6.2% (95%CI, 5.2 to 7.3; $P < 0.001$). This pattern is unique. All current treatments improve spine BMD (largely trabecular) more than femoral neck BMD (largely cortical). These results suggest that NTG has uniquely potent effects on cortical bone mass that may translate into greater effects on the risk of nonvertebral fractures. At the radius and the tibia, respectively, 2 years of NTG increased cortical thickness (13.9 and 24.6%), cortical area (10.6 and 10.0%) and periosteal circumference (7.4 and 2.9%), with small effects on cortical density. This pattern indicates that nitroglycerin increases cortical bone mass; the increase in periosteal diameter suggests that it may induce formation of new bone on the periosteal surface, a biologically unique effect not observed with antiresorptives. As would be expected from these effects on cortical bone, NTG increases indices of strength: section modulus or bone bending (10.7 and 9.8%), and polar moment of inertia or bone twisting (7.3 and 14.5%) at the radius and tibia respectively. These improvements in bone strength indicate that NTG should decrease the incidence of nonvertebral fractures. Our findings are different from what has been reported from a randomized trial of once-daily NTG ointment (Nitro-Bid 22.5 mg); that study did not find increased BMD at the lumbar spine, femoral neck of total hip. However, adherence to treatment was poor.[42]

Mechanism of Action

As the molecular targets for NO action in bone cells have not yet been elucidated, the mechanism by which nitrates influence bone remains unclear. Nitrates may influence bone cells indirectly by acting as NO donors and inducing local vasodilation—similar to the effects of mechanical loading on bone whereby fluid shear stress activates G-protein-linked mechanoreceptors in osteocytes, causing NO and other second messengers to be released, which then act as intermediaries signaling to osteoblasts and osteoclasts to form or resorb bone.[43–45] Alternatively (or in addition), nitrates may have more direct effects on osteoclasts and osteoblasts. For example, NO donors may have a beneficial effect on bone by inhibiting secretion of sclerostin. Sclerostin which is secreted by osteocytes, antagonizes osteoblast differentiation and mineralization and increases mature osteoblast apoptosis by inhibiting the *Wnt* pathway.[46] Individuals with congenital absence or reduction of circulating levels of sclerostin have very high bone mass.[47] We examined the effect of organic nitrates (NTG ointment) on sclerostin in a subset of subjects (25 in the placebo group and 25 in the treatment group) participating in our double-blind, placebo-controlled randomized trial that had as it primary aims to assess the effects of NTG on bone turnover, density, geometry and strength; note that the findings from this trial have been discussed earlier.[41] Circulating sclerostin levels were measured by the Biomedica ELISA assay (Biomedica, Durham, NC, USA) in a random sample of 25 women in the treatment group and 25 women in the placebo group at baseline and at year 2. Circulating sclerostin levels decreased significantly in the nitroglycerin-treated participants by 21% (baseline, 32.8 pg l^{-1}; year 2, 25.6 pg l^{-1}; $P = 0.01$) but did not change in the placebo group (baseline, 26.7 pg l^{-1}; year 2, 24.6 pg l^{-1}, $P = 0.30$). The percent decrease in circulating sclerostin levels after 2 years differed significantly between the treatment and placebo groups (-21 vs -6%, $P < 0.001$).[48] These results suggest that nitroglycerin may increase bone mass in postmenopausal women by decreasing sclerostin production.

Conclusions

The number of osteoporotic fractures is increasing worldwide as populations age. Treatments for osteoporosis are limited by cost, side effects and most importantly efficacy. Current treatments decrease vertebral fractures (which consist of trabecular bone) but have very limited effects on cortical bone—yet most osteoporotic fractures occur at these sites (for example, the hip, legs, forearm and upper arm). There is a need for easily administered, inexpensive, well-tolerated agents that increase bone cortical strength and substantially decrease the risk of fractures. Our data suggest that organic nitrates may meet this need.

A randomized trial with fracture endpoints is essential to establish the efficacy of nitrates for clinical use; particular emphasis should be placed on studying the effects of organic nitrates in women previously taking antiresorptive agents—it may be that the bone turnover response to nitrates may be blunted after treatment with antiresorptives. To design and conduct a fracture-prevention trial requires choosing a preparation and dose of nitrate that maximizes its beneficial effects on bone while minimizing adverse effects (headaches); a dose and formulation study. We are currently in the process of conducting such a study. Once we have identified the ideal dose and formulation of nitrate—that is, the nitrate that gives the least headaches, is easy to use, and is associated with good effects on bone turnover—we plan to conduct and execute a large randomized controlled trial to determine if nitrates can reduce fractures.

Conflict of Interest

The authors declare no conflict of interest.

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