Zoledronic not only Inhibits Osteoclast Activity but also Enhances Mineralisation

INTRODUCTION:
Zoledronic acid is a potent anti-resorptive agent used in the treatment of osteoporosis to reduce fracture risk [1]. The effects of bisphosphonates, such as zoledronic acid, on bone are the result of two key properties: their affinity for bone mineral and their inhibitory effects on osteoclasts [2]. Evidence also suggests that bisphosphonates may directly affect the proliferation and differentiation of osteoblasts [3,4]. However it is not yet known whether this change in osteoblast activity leads to osteogenesis. The aim of this study was to identify whether zoledronic acid has an anabolic effect on osteogenesis both in vitro and in vivo. Cell culture experiments determined the effects of zoledronic acid on cell proliferation and osteogenesis. A second series of experiments examined the in vivo influence of zoledronic acid on gene expression and matrix composition in an ovine model of osteoporosis.

METHODS:

In vitro study: Rat mesenchymal stem cells (MSC) were maintained in culture for up to 28 days. 5x10^5 cells were cultured in regular growth media (α-MEM, 10% FBS, 2% penicillin-streptomycin, 1% L-glutamine, 1% Glutamax and 1% non essential amino acids), which was supplemented with either 0.05, 0.1 or 0.2μg of zoledronic acid (Z1, Z2 and Z3 respectively; Alpha Technologies Ltd) or left unsupplemented. A positive osteogenic control was included, in which cells were cultured in α-MEM, 10% FBS and 2% penicillin-streptomycin with 100μM dexamethasone, 50μg ascorbic acid and 10μM β-glycerophosphate. Cells were fixed after 7, 14, 21 and 28 days. Cell number was determined using DAPI while alizarin red stained mineralised nodules and this was quantified.

In vivo study: Under animal license and with institution ethical approval, fourteen skeletally mature (4+ years) ewes were randomly assigned into a twelve (n=5) or thirty (n=9) month ovariectomy (OVX) group, at which point the animals were euthanised. Twenty months post-OVX, four OVX animals were randomly selected from the latter time-point to receive 25mg of zoledronic acid administered over a 5 week period (Zol; Novartis Pharma).

Real time reverse transcription polymerase chain reaction (Real time RT-PCR): mRNA was extracted from the right metacarpal (Totally RNA Kit, Applied Biosystems). First-strand cDNA synthesis was performed with 1 μg total RNA from each sample. Gene expression was analysed by real time RT-PCR, using BioRad iQ SYBR Green Supermix on a Rotor-Gene thermocycler (Corbett Research, Australia). Receptor activator of nuclear factor Kappa B ligand (RANKL), osteoprotergine (OPG), osteopontin (OPN) and osteocalcin (OCN) expression remained elevated subsequent to zoledronic acid treatment.

Fourier-Transform Infrared Spectroscopy (FTIR): Powdered bone samples from the right metacarpal were placed into a Tensor 27 FTIR machine (Bruker Optik GmbH) and spectra obtained at a resolution of 4 cm⁻¹ from 400 to 4000 cm⁻¹. Mineral-to-matrix ratio and crystallinity were calculated.

Statistica: mRNA expression data was analysed using a fully nested ANOVA. In vitro data and FTIR data were analysed using ANOVA and Mann-Whitney rank sum tests. p≤0.05 was considered significant.

RESULTS:

The positive osteogenic group showed the highest mineralisation levels (p<0.01). At the lowest zoledronic acid concentration, Z1, there was a significant increase in mineralisation compared to higher concentrations and the MSC controls for days 14, 21 and 28 (Figure 1).

Zoledronic acid had a sustained negative effect on cell proliferation over time. At the initial time point there was no difference in cell number, however, continued supplementation with zoledronic acid resulted in a significant reduction in cell number with greater losses seen at higher concentrations (p<0.05).

Consistent with the known mode of action of bisphosphonates, the inhibition of osteoclast activity, the current study found a significant reduction in the RANKL:OPG ratio in those animals treated with zoledronic acid (p<0.001). Similarly osteopontin (OPN) was also reduced following treatment (Figure 2). In contrast, osteocalcin (OCN) expression remained elevated subsequent to zoledronic acid treatment.

DISCUSSION:
This study aimed to determine whether zoledronic acid had the potential to not only inhibit osteoclast activity but also to promote osteogenesis and mineralisation by osteoblasts. Through the use of both in vitro cell culture experiments and an in vivo study we have shown an inverse dose relationship between zoledronic acid and mineralisation in MSCs and also changes in the genes responsible for mineralisation and corresponding changes in bone composition.

The in vitro study demonstrated the potential that zoledronic acid has to promote mineralization in the absence of osteogenic factors. Zoledronic acid can promote differentiation of MSCs down an osteogenic lineage and promote mineralisation. However, sustained use of zoledronic acid in cell cultures does result in a reduction in cell number thus demonstrating the importance of the dose of the drug being used and the duration of treatment.

Osteopontin also plays a role in regulating osteoclastogenesis. However, there is conflicting data on whether osteopontin levels are decreased or elevated following bisphosphonate treatment. The current study found a reduction in expression of the osteopontin gene following zoledronic acid treatment, which is encouraging as over-expression is a risk factor for osteoporosis. Uncertainty also surrounds whether osteocalcin levels are reduced or increased following bisphosphonate treatment. As osteocalcin is important for mineralisation, the increase in expression of the osteocalcin gene, measured here, affirms the potential that zoledronic acid has to promote mineralisation.

While no significant changes were measured by FTIR, the changes seen were encouraging. There was a strong trend towards an increase in mineral-to-matrix ratio and also the mineral crystallinity or maturity.

In conclusion this study has demonstrated that zoledronic acid not only inhibits osteoclast activity but also enhances mineralisation.

SIGNIFICANCE:
This study provides further evidence of the mechanism by which zoledronic acid reduces fracture risk in osteoporosis. It also demonstrates the importance of drug dose and the duration of exposure.

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