Bones and Beyond. Bisphosphonates celebrating 50 years. Can osteoporosis treatments prolong health span and life span?

La Hulpe 15th March 2019

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Sir Edward Mellanby
Towards a molecular explanation of actions of bisphosphonates

~29,000 publications in PubMed

Risedronate in Farnesyl Pyrophosphate Synthase


Davos
Chemical Relationships.  
Phosphates, Pyrophosphate and Bisphosphononates

Inorganic Phosphate (Pi)

\[
\text{HO - P = O} \\
\text{OH}
\]

Inorganic Polyphosphate as acid, where \( n \) = 1 to 100+. (eg Graham salt)

Chemical water softener

Inorganic Pyrophosphate (PPi)

Chemically and Enzymatically Labile

Nature’s “water softener”

Bisphosphononate (BP) as acid

Chemically Stable

Used as medicines
Clinically Utilised Bisphosphononates.

Different Mechanisms of Action

- **Early BPs**: non-nitrogen containing
  - Etidronate
  - Clodronate
  - Tiludronate

- **“Second” generation**: nitrogen-containing with short alkyl chains
  - Pamidronate
  - Neridronate
  - Alendronate (Fosamax)

- **“Third” generation**: (from medicinal chemistry optimisation): nitrogen-containing with branched or ring structure
  - Ibandronate
  - Zoledronate
  - Minodronate
  - Risedronate
  - Ox-14, a new BP

- N-BPs inhibit FPPS enzyme in mevalonate pathway
The birth of bisphosphonates
Science Vol 165, 1969

Diphosphonates Inhibit Hydroxyapatite Dissolution in vitro and Bone Resorption in Tissue Culture and in vivo

Abstract. Two diphosphonates containing the P–C–P bond, Cl₂C(PO₂HNa)₂ and H₃C(PO₂HNa)₂ retard the rate of dissolution of apatite crystals in vitro. They inhibit bone resorption induced by parathyroid extract in mouse calvaria in tissue culture and in thyroparathyroidectomized rats in vivo.

Diphosphonates Inhibit Hydroxyapatite Dissolution in vitro and Bone Resorption in Tissue Culture and in vivo

Abstract. Two diphosphonates containing the P–C–P bond, Cl₂C(PO₂HNa)₂ and H₃C(PO₂HNa)₂ retard the rate of dissolution of apatite crystals in vitro. They inhibit bone resorption induced by parathyroid extract in mouse calvaria in tissue culture and in thyroparathyroidectomized rats in vivo.

Inorganic pyrophosphate inhibits the precipitation (1, 2) and the dissolution (3) of hydroxyapatite crystals in vitro. Because pyrophosphate is present in plasma (4), teeth (5), and bone (6), we have suggested (2) that it might regulate both the formation and destruction of mineralized tissues in vivo. Although pyrophosphate and longer-chain condensed phosphates can inhibit the precipitation of calcium phosphate in chick embryo femurs in tissue culture (7) and in the aorta (8) and skin (9) of the rat, it has not yet been possible to demonstrate an effect on the resorption of living bone. This failure to influence bone resorption may be due to hydrolysis of the P–O–P bond locally in the bone by pyrophosphatase before it can reach its site of action.

Substances were therefore sought that would be related in structure to pyrophosphate but resistant to chemical and enzymatic hydrolysis. Various compounds containing the P–C–P bond have been synthesized and found to have an effect similar to that of condensed phosphates on the precipitation of hydroxyapatite in vitro and on calcification in vivo (10). In addition, they were also active when administered orally (11). We now describe the effect of two such compounds containing a P–C–P bond, namely, sodium dichloromethylenebisphosphonate [Cl₂C(PO₂HNa)₂] and sodium methylenebisphosphonate [H₃C(PO₂HNa)₂], on the dissolution of apatite crystals in vitro and on bone resorption induced by parathyroid extract in tissue culture and

To our knowledge the diphosphonates are still the only substances, apart from thyrocacitonin (14), that can significantly inhibit bone resorption in vivo, although fluoride (15), orthophosphate (16) and estrogens (17) have been studied for their potential therapeutic effect in this respect. Since diphosphonates appear to be relatively nontoxic they might prove valuable in the treatment of osteoporosis and other human diseases that involve increased resorption of bone.

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Early Clinical Use of Bisphosphonates For Bone Imaging

Technetium-99m-labelled-MHBP
Bisphosphonates Are Taken Up Avidly By Bone

BP = bisphosphonates

Bind to bone mineral

Concentrate around and inside osteoclasts at sites of bone resorption

Bone

Technetium-99m-labelled BP locates selectively to cancer sites in bone

Image from Fraser Coxon

- Bisphosphonate on bone surface and inside osteoclast
- Osteoclast membrane

The bisphosphonates have tissue selectivity for bone
Bisphosphonates Act by Inhibiting the Mevalonate Pathway in Osteoclasts

Active Osteoclast | Inactive Osteoclast | Apoptotic Osteoclast

HMG Co-A → Mevalonate → Farnesyl-PP

N-Bisphosphonates inhibit Farnesyl pyrophosphate synthase

Geranylgeranyl-PP reduced

Isoprenylation of GTP-binding proteins (Rab, Rho, Ras, Rac etc)

From Rogers, Reska, Russell 2002
Mevalonate pathway. Multiple sites of inhibition by N-BPs
Fluorescent risedronate binds to osteocyte lacunar walls

**Osteocytes that are becoming entombed**

- Green: FAM-risedronate
- Blue: Nuclei
- Red: wheatgerm-agglutinin

**Images from Fraser Coxon**

**Osteocytes in cortical bone close to vascular channels**

- Green: FAM-risedronate
- Blue: Nuclei

**Bisphosphonates have prosurvival effects on osteocytes through extracellular mechanisms**

Plotkin et al 2002, J Biol Chem

**Diagram**

- Bisphosphonates
- Cx43 hemichannel
- SH2 SH3 Src
- MEK
- ERKs
- Survival
Bisphosphonates are Used to Treat Many Bone Resorption Disorders

Paget’s Disease
Myeloma
Bone metastases
Osteoporosis
Etidronate in Paget’s Disease

The Nuffield Orthopaedic Centre

Smith Russell & Bishop, Lancet 1971
Bisphosphonates are Used to Prevent Fractures In Osteoporosis

Wrist
Spine
Hip

Oral “blockbuster” BPs

Alendronate

Risedronate

Ibandronate

Zoledronate is given once yearly iv
Efficacy of anti-osteoporosis treatments on hip fractures

Adapted from Reid IR. Nat Rev Endocrinol 2015;11:418-28.
Zoledronate has a remarkably long duration of action!

CTX is a biomarker of bone resorption

Osteopenic Women
Given just 1 Infusion of Zoledronate 5mg

PINP is a biomarker of bone formation

Bisphosphonates (esp Zoledronate) Are Used to Prevent Skeletal Related Events (SREs) In Cancer (Hypercalcaemia, Bone Loss And Fractures etc)

- Skel with lytic lesions in myeloma
- Humerus with lytic lesions in breast cancer

![Zoledronate](image)

Zoledronate (iv)

![Pamidronate](image)

Pamidronate (iv)
MRC Myeloma IX: Early Survival Benefits of Zoledronic Acid

HR = 0.64 (95% CI = 0.47, 0.86)  
\( P = .0044 \)

HR = 0.76 (95% CI = 0.58, 0.98)  
\( P = .04 \)

Number at risk:
ZOL 981 972 958 949 944 935 927 920 910
CLO 979 965 952 937 924 913 898 883 871

Number at risk:
ZOL 981 972 958 946 937 923 907 892 875
CLO 979 963 945 928 911 893 875 857 843

Abbreviations: CI, confidence interval; CLO, clodronate; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; ZOL, zoledronic acid.

Examples of Potential Non-Skeletal Effects of Bisphosphonates
(derived from clinical observations)

- Cancer prevention and treatment
- Reducing colon cancers
- Reducing heart attacks and heart failure
- Increasing survival in intensive care units
- Preventing radiation damage by enhancing DNA repair and tissue regeneration.
- Reducing mortality and extending life span ("senolytics")
Reduced Risk Of Colon Cancer Death In Patients Treated With Alendronate - Danish National Register Based Cohort Study.

- 33,011 osteoporosis patients, mean age 71.3 years, began alendronate 1996-2005
- 66,022 matched control subjects
- Mean follow-up 4.9 years
- 629 colon cancer deaths – 39% reduction in Aln users
- Overall mortality reduction 17%

Pazianas M, Abrahamsen B, Eiken PA, Eastell R, Russell RG

Osteoporosis Int 2012

39% reduction in deaths from colon cancer
Effects of Bisphosphonates on Life Span, Health Span and Mortality
Zoledronate Reduced Risk of All-cause Mortality by 28% Over Time in Hip Fracture Trial

Hazard ratio, 0.72 (95% CI, 0.56–0.93)
P=0.0117
Absolute risk reduction, 3.7%

Effect Of Statins And Bisphosphonates In A Model Of Human Premature Aging

Inhibition of prenylation of progerin and preLamin A increases life span

Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging

Varela et al  Nature Medicine, 2008
Accelerated Ageing
Hutchinson-Gilford Progeria Syndrome (HGPS).

HGPS is due to a genetic defect in prenylation.

Life span in mouse model is doubled by giving zoledronate plus a statin.

Clinical trials in progress.
**Reduced Mortality with Osteoporosis Treatment**

![Graph showing the effect of different osteoporosis treatments on mortality](image)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>Relative Risk [95% Confidence Interval]</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alendronate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black 1996</td>
<td>24/1022</td>
<td>21/1005</td>
<td>1.12 [0.63, 2.01]</td>
<td>3</td>
</tr>
<tr>
<td>Cummings 1998</td>
<td>37/2214</td>
<td>40/2218</td>
<td>0.93 [0.59, 1.44]</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>61/3236</td>
<td>61/3223</td>
<td>1.00 [0.70, 1.41]</td>
<td>P=0.98</td>
</tr>
<tr>
<td><strong>Risedronate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harris 1999</td>
<td>15/813</td>
<td>16/815</td>
<td>0.94 [0.47, 1.89]</td>
<td>2</td>
</tr>
<tr>
<td>Reginster 2000</td>
<td>11/407</td>
<td>17/407</td>
<td>0.65 [0.31, 1.36]</td>
<td>2</td>
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<tr>
<td>McClung 2001</td>
<td>114/3162</td>
<td>127/3184</td>
<td>0.90 [0.71, 1.16]</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>140/4382</td>
<td>160/4406</td>
<td>0.88 [0.70, 1.10]</td>
<td>P=0.27</td>
</tr>
<tr>
<td><strong>Strontium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meunier 2004</td>
<td>29/826</td>
<td>21/814</td>
<td>1.36 [0.78, 2.37]</td>
<td>3</td>
</tr>
<tr>
<td>Reginster 2005</td>
<td>142/2526</td>
<td>159/2503</td>
<td>0.88 [0.71, 1.10]</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>171/3352</td>
<td>180/3317</td>
<td>0.94 [0.77, 1.15]</td>
<td>P=0.54</td>
</tr>
<tr>
<td><strong>Zoledronic acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black 2007</td>
<td>130/3862</td>
<td>112/3852</td>
<td>1.16 [0.90, 1.48]</td>
<td>15</td>
</tr>
<tr>
<td>Lyles 2007</td>
<td>101/1054</td>
<td>141/1057</td>
<td>0.72 [0.56, 0.91]</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>231/4916</td>
<td>253/4909</td>
<td>0.90 [0.76, 1.08]</td>
<td>P=0.26</td>
</tr>
<tr>
<td><strong>Denosumab</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cummings 2008</td>
<td>70/3902</td>
<td>90/3906</td>
<td>0.78 [0.57, 1.06]</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>70/3902</td>
<td>90/3906</td>
<td>0.78 [0.57, 1.06]</td>
<td>P=0.11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>673/19788</td>
<td>744/19761</td>
<td>0.90 [0.81, 1.00]</td>
<td>P=0.044</td>
</tr>
</tbody>
</table>

Test for heterogeneity: $I^2 = 23\%, P=0.23$

*Bolland et al 2010 JCEM 95:1174-81*
Survival After Fracture, With or Without Osteoporosis Therapies - The Dubbo Study

Center J, et al. JCEM 2011
Bisphosphonates Reduce the Risk of Myocardial Infarction in Patients with Rheumatoid Arthritis

- National Databank for Rheumatic Diseases, prospective study of RA patients, 2002-2011
- n=19,281. Number of patients ever on bisphosphonate: 5,891
- HR for MI among treated patients 0.72 (0.54-0.96) when on BP therapy compared to when on no therapy

Reduction of in-hospital mortality in patients who were treated with bisphosphonate prior to ICU admission

*J Clin Endocrinol Metab.* 2016
*May;*101(5):1945-53

**Preadmission bisphosphonate and mortality in critically ill patients**

Paul Lee¹,²,³,⁴, Carmen Ng², Anthony Slattery¹, Priya Nair³,⁴, John A. Eisman¹,⁵,⁶,⁷, Jacqueline R. Center¹,⁶,⁷

Department of Endocrinology¹, Pharmacy Department², Intensive Care Unit³, PET and Nuclear Medicine⁴, St Vincent’s Hospital; Diabetes and Metabolism Division³, Bone Biology Division⁵, Garvan Institute of Medical Research, Faculty of Medicine⁶, University of New S Wales, School of Medicine University of Notre Dame, Sydney, NSW, Australia

- 7830 critically ill patients admitted to Intensive Care Unit (ICU) between 2003 and 2014.
- 245 patients received preadmission bisphosphonate.
- Bisphosphonate users were older (66±16 vs. 58±18 years, p<0.01) and had greater co-morbid disease burden (Charlson co-morbidity index: 5.7±3.6 vs. 4.6±3.8, p<0.01), yet bisphosphonate use was associated with a lower in-hospital mortality [Mortality Rate Ratio (MRR): 0.41 (95% CI 0.24–0.71, p<0.01)].

**Figure 2. Bisphosphonate and survival** Survival curves comparing bisphosphonate users and nonusers.

59% reduction of in-hospital mortality in patients who were treated with bisphosphonate pre-ICU admission
Association of Alendronate and Risk of Cardiovascular Events in Patients With Hip Fracture

- Hong Kong study of 34,991 patients with newly diagnosed with hip fracture from 2005 through 2013.
- 4594 BP treated patients were matched with 13,568 nontreated patients.
- 67% reduction in CVD mortality at 1 yr.
- 45% reduction in Myocardial Infarcts (MI) at 1 yr.
- ~28% reduction in strokes at 5 & 10 yrs

Mortality Outcomes
2010 meta-analysis of 10 trials

RR = 0.90 (0.81–1.00)*

*Borderline statistical significance
New Data on Zoledronate in Osteopenia


- RCT in 2000 women, age >65 years, hip T-score -1.0 to -2.5.
- 4 infusions of zol 5mg or saline every 18 months
- Vitamin D supplements monthly, no calcium supplements
- Trial duration 6 years
- Zoledronate prevents fractures in osteopenia
- Reductions in cancer and vascular disease
- Trend to lower mortality
New Data on Zoledronate in Osteopenia

Reid et al NEJMEd 2018

- Zoledronate prevents fractures in osteopenia
- 18-month dose interval is effective
- Ca supplements not needed
- Baseline variables do not impact on efficacy
- Reductions in cancer and vascular disease
- Trend to lower mortality
Reduction in Osteoporotic/Fragility Fractures after Zoledronate for 6 years

HR 0.63, P < 0.0001

NNT = 15

Fracture Reduction with Zoledronate over 6 years

Mortality Reduction after Zoledronate for 6 years

OR = 0.65 (0.40, 1.046)

Prespecified Adverse Events of Interest.
Effects of zoledronate on cancer, cardiovascular events and survival.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Placebo (N=1000)</th>
<th>Zoledronate (N=1000)</th>
<th>Odds Ratio with Zoledronate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events no.</td>
<td>Events no.</td>
<td>Events no.</td>
</tr>
<tr>
<td></td>
<td>Events per 1000 Woman-Yr (95% CI)</td>
<td>Women with at Least One Event</td>
<td>Events per 1000 Woman-Yr (95% CI)</td>
</tr>
<tr>
<td>Death</td>
<td>41 (5.4–9.4)</td>
<td>27 (3.0–6.6)</td>
<td>0.65 (0.40–1.05)</td>
</tr>
<tr>
<td>Sudden death</td>
<td>1 (0.002–0.9)</td>
<td>3 (0.1–14.8)</td>
<td>3.01 (0.3–28.9)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>43 (5.3–9.8)</td>
<td>25 (2.7–6.2)</td>
<td>0.61 (0.36–1.02)</td>
</tr>
<tr>
<td>Coronary-artery revascularization</td>
<td>32 (3.7–7.7)</td>
<td>23 (2.5–5.8)</td>
<td>0.72 (0.41–1.27)</td>
</tr>
<tr>
<td>Stroke</td>
<td>22 (2.3–5.7)</td>
<td>20 (2.1–5.2)</td>
<td>0.85 (0.44–1.63)</td>
</tr>
<tr>
<td>Composite of vascular events*</td>
<td>98 (13.5–20.3)</td>
<td>71 (9.3–15.1)</td>
<td>0.76 (0.52–1.09)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>15 (1.4–4.2)</td>
<td>24 (2.6–6.0)</td>
<td>1.66 (0.85–3.24)</td>
</tr>
<tr>
<td>Cancer†</td>
<td>127 (18.0–18.1)</td>
<td>87 (11.7–18.1)</td>
<td>0.67 (0.50–0.89)</td>
</tr>
<tr>
<td>Osteonecrosis of the jaw</td>
<td>0</td>
<td>0</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>92 (12.6–19.1)</td>
<td>88 (11.9–18.3)</td>
<td>0.98 (0.67–1.44)</td>
</tr>
</tbody>
</table>

Possible Mechanisms to explain effects of Bisphosphononates on Life Span and Mortality

- Reducing leaching of toxins from bone. eg Pb, Cd, Hg etc
- Effects on lipids
- Effects on vascular calcification
- Effects on γδT cells, ‘acute phase response’
- Effects on stem cells & ageing
- Effects on cell senescence, autophagy etc
- Protection from tissue damage, irradiation,
- Enhanced DNA repair and tissue regeneration.
- Also effects in cancers to reduce mortality
Osteogenic contributions to arteriosclerotic disease

Adapted from Towler DA Circulation 2017

Morphogens and paracrine signals
- BMPs
- Wnt/Dkk
- Shh/Ihh
- Notch
- PTHrP
- VEGF
- Ang-II
- Adenosine

Inflammatory cues/Innate immunity
- TNF, IL1, IL6
- RAGE ligands
- Oxylipid TLR ligands
- ER stress response

Ectoenzymes
- Alkaline phosphatase
- CD73
- ENPP1
- Cathepsins
- MMPs

Hormones
- PTH
- 1,25(OH)2D3
- Estradiol
- Testosterone
- Aldosterone
- Platelet-derived TGFβ
- Insulin
- GLP1

Extracellular matrix and vesicles
- Collagens I/II/III/X/XI
- Proteoglycans
- Annexins
- Sialoproteins
- Phospholipids
- Phospholipases

Transcription factors
- Runx2
- Msx1, Msx2
- Sox9, Sox2
- Smad family
- Rel domain family
- HIF1α
- Nuclear receptors

Mineralization inhibitors
- Fetuin
- Osteopontin
- Pyrophosphate
- Bisphosphonates?
- MGP
Zoledronate can protect human mesenchymal stem cells from radiation damage and cellular ageing.
Unusual uses of Bisphosphonate

• Herbicides
• Anti-microbial (microbiome effects?)
• Anti/protozoan parasites eg.
  – Entamoeba
  – Cryptosporidium
  – Leishmaniasis
  – Trypanosomes
  – Plasmodium (malaria)
Using Bisphosphonates for Targeting Drugs to Bone

- Estrogens
- Prostaglandins
- Anti-biotics
  - Ciprofloxacin
- Anti-cancer drugs
  - Bortezomib
Bisphosphonates

Historical Highlights

- BPs used as pyrophosphate analogues to inhibit calcification
- Bisphosphonates inhibit bone resorption
- Could be given orally as well as parentally
- Use in bone imaging Tc99m-BPs
- Effective in Paget’s disease
- Effective in hypercalcaemia of malignancy
- Reduce skeletal related events in breast, prostate and other cancers, and in myeloma
- Reduce fractures in osteoporosis
- Safe and effective leading drugs, but rare side effects get media attention
- Other effects
  - Healthspan, life span
  - Cardiovascular benefits
  - Use for drug delivery
Bisphosphonates. 50\textsuperscript{th} Birthday!

bisphosphonates\textsuperscript{2019}
CELEBRATING 50 YEARS

15-17 JULY 2019
SHEFFIELD, UK

In association with the Mellanby Centre, University of Sheffield

www.bisphosphonates2019.org