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New insights into the molecular actions of nitrogen-containing bisphosphonates

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About 10 years ago it was found that mammalian cells could convert some bisphosphonates (BPs) intracellularly into methylene-containing (AppCp-type) analogues of ATP, that accumulate in the cytosol,[1,2] reviewed recently.[3] Only simple BPs (ie those that most closely resemble PPi, such as clodronate and etidronate, but not alendronate, pamidronate or other N-BPs) appear to be metabolised.[2] More recently, we have confirmed that osteoclasts *in vivo* are capable of metabolising clodronate to AppCCl2p,[4] and shown that the accumulation of the AppCCl2p metabolite of clodronate in osteoclasts *in vitro* inhibits bone resorption by inducing osteoclast apoptosis,[4] perhaps by inhibiting the adenine nucleotide translocase (ANT), a component of the mitochondrial permeability transition pore.[5] Nitrogen-containing bisphosphonates (N-BPs), which are several orders of magnitude more potent at inhibiting bone resorption *in vivo* than the simple BPs,[6,7] are not metabolised[2] but are potent inhibitors of farnesyl diphosphate synthase (FPP synthase), a key enzyme of the mevalonate pathway. This enzyme is inhibited by nanomolar concentrations of N-BPs.[8-10] Zoledronic acid, and the structurally similar minodronate, are extremely potent inhibitors of FPP synthase[10] and inhibit enzyme activity even at picomolar concentrations. Importantly, studies with recombinant human FPP synthase revealed that minor modifications to the structure and conformation of the R^2 side chain that were known to affect anti-resorptive potency on osteoclasts[10] or activation of γ,δ-T cells[11] also affect the ability to inhibit FPP synthase (reviewed recently;[6] thus finally explain the relationship between BP structure and anti-resorptive potency. Furthermore, these studies strongly suggested that FPP synthase is the major pharmacologic target of N-BPs in osteoclasts and other cell types.

The exact mechanism by which N-BPs inhibit FPP synthase is only just becoming clear. The recent generation of an x-ray crystal structure of the human FPP synthase enzyme, co-crystallised with risedronate or zoledronic acid,[12,13] indicates that the BPs appear to bind in one of the two isoprenoid lipid binding pockets in the enzyme active site (that would normally bind GPP or DMAPP), with the R^2 side chain positioned in the hydrophobic cleft that normally accomodates an isoprenopid lipid, and the phosphonate groups bound to a cluster of 3 magnesium ions. Interestingly, the nitrogen atom in the R^2 side chain appears to form hydrogen bonds with a critical, conserved threonine residue. This is consistent with the earlier suggestion by Oldfield and colleagues that N-BPs mimick the structure of the enzyme's natural isoprenoid pyrophosphate substrates GPP/DMAPP and act as carbocation transition state analogues or reactive intermediates,[14] competing for binding at the GPP/DMAPP substrate binding pocket in the active site of the enzyme. N-BPs also appear to inhibit bacterial FPP synthase in a similar manner.[15]

Enzyme kinetic analysis with human FPP synthase indicates that the interaction with N-BPs is highly complex and characteristic of *slow-tight binding* inhibition. Initially, N-BPs appear to compete directly with DMAPP or GPP for binding to the DMAPP/GPP binding pocket. However, this is followed by more complex interactions that affect the binding of IPP. Recent computer modelling studies suggested that a second molecule of N-BP might also bind to the (IPP) isoprenoid lipid pocket in the enzyme, which appeared to be supported by enzyme kinetic analysis showing biphasic modes of inhibition.[16] However, binding of N-BP to the IPP pocket of FPP synthase has not yet been confirmed in x-ray crystal structures,[12,13] and the kinetic studies suggesting inhibition at the IPP site may reflect more complex conformational changes in the structure of the enzyme due to binding of N-BP in the DMAPP/GPP pocket. Nevertheless, these studies are beginning to key insights into the reasons why minor changes to the structure of the N-BP side

chain or to the phosphonate groups markedly influence the potency of N-BPs for inhibiting FPP synthase, hence explaining at the detailed molecular level the structure-activity relationships of N-BPs for inhibiting bone resorption and for activating γ,δ-T cells, which are both due to inhibition of FPP synthase.[6,11,17]

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