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Targeting therapeutics to bone by conjugation with bisphosphonates

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Bisphosphonates target and bind avidly to the mineral (hydroxyapatite) found in bone. This targeting ability has been exploited to design and prepare bisphosphonate conjugate prodrugs to deliver a wide variety of drug molecules selectively to bones. It is important that conjugates be stable in the blood stream and that conjugate that is not taken up by bone is eliminated rapidly. The prodrugs should release active drug at a rate appropriate so as to provide efficacy. Radiolabelling is the best method to quantify and evaluate pharmacokinetics, tissue distribution, bone uptake and release of the active drug(s). Recent reports have described bisphosphonate conjugates derived from the antiresorptive drug, alendronic acid and anabolic prostanoid drugs that effectively deliver prostaglandins and prostaglandin EP4 receptor agonists to bone and show enhanced anabolic efficacy and tolerability compared to the drugs alone. These conjugate drugs can be dosed infrequently (weekly or bimonthly) whereas the free drugs must be dosed daily.

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Introduction

Treating diseases of the bone can be difficult due to relatively low vascularization and the physical barriers of penetrating the tissue. High doses of systemic drugs may be necessary to provide efficacy in bone and in the case of drugs with important systemic effects or side effects these may limit or exclude their use in treating conditions of the bones. Many researchers in the past have attempted to identify chemical structural elements that may preferentially bind to the hydroxyapatite mineral in bone as a way to target drugs to bone [1,2•]. Some polyhydroxy containing molecules such as tetracyclines [3] or polyacidic peptides [4,5], polymers [6,7], hydroxylated heterocycles [8] and monophosphonates [9] have been found to bind to apatite and have been studied but binding to hydroxyapatite may impair or block biological activity of such compounds. One structure type that has found considerable success as a bone targeting moiety is the class of drugs known as bisphosphonates. Bisphosphonates and hydroxy-bisphosphonates represent non-hydrolyzable analogs of pyrophosphate and bind avidly to hydroxyapatite. Once complexed to hydroxyapatite the bisphosphonates are essentially irreversibly bound and can only be liberated in the course of bone turnover by osteoclasts, cells that dissolve the mineral in bone with hydrochloric acid as part of the bone resorption process [10]. Aminoalkyl 1-hydroxy-1,1-bisphosphonates such as alendronic acid (Fosamax™) are effective antiresorptive drugs and have been in wide use for decades [11]. Bisphosphonates have been used as a vehicle to selectively deliver proteins [12], liposomes [13], nanoparticles [14] and small molecules to bone by virtue of their bone binding properties and can serve as a point of attachment for a wide variety of drugs and active molecules including proteasome inhibitors [15], anticancer drugs [16,17], analgesics [18] and antibiotics [19–21].

Bisphosphonates such as alendronate are very polar molecules and are poorly absorbed following oral dosing and are rapidly eliminated from the body if they do not bind first to bone [22–24]. Radiolabelling studies have shown that 40–50% of alendronate is taken up by bones after systemic dosing [23]. Conjugates with bisphosphonates are expected to also not be well absorbed and are usually studied after IV dosing.

An ideal drug-bisphosphonate prodrug for bone-targeted delivery would have the following properties:

(1) Be tethered by a linker element that is stable in the blood stream such that within a short time (< 1 day) the molecule will either bind to bone intact or be largely eliminated intact from the body.
(2) Be inactive in so far as the activity of the drug to be delivered is concerned.
(3) Once bound to bones, release the active drug in a slow and sustained manner. An ideal half time for release of about 4–7 days would support infrequent dosing of once a week or twice a month.
(4) Be sufficiently potent so that the overall dose of conjugate (and body load of bisphosphonate in bones)

would be relatively low and within the scale of doses of bisphosphonates that have been shown to be safe in the past.

(5) The drug to be delivered is on its own poorly distributed to bone, or if dosed as a free drug in the systemic circulation, exhibits unacceptable side effects that limit its use.

To quantitatively assess stability and integrity of a drug-bisphosphonate conjugate prodrug it is preferrable to radiolabel the drug (and also, ideally, the bisphosphonate or linker component). The radiolabel(s) can then be used to quantitatively assess tissue distribution, bone uptake and elimination after dosing, and to demonstrate release of the bone-bound drug to determine the half-time for drug release. While many researchers have designed and synthesized bisphosphonate-drug conjugates and some have evaluated their effects *in vivo* (*vide supra*), very few have evaluated biodistribution and elimination of the prodrug *in vivo* or quantified bone exposure or drug release in the bones.

One class of drugs that have been successfully targeted to bones both via bisphosphonates (and also via conjugation to polyanionic polymers [6]) are the bone anabolic prostaglandins related to prostaglandin E_2 (PGE_2). PGE_2 was shown to be anabolic for bone in animals [25,26] and in humans [27]. However, PGE_2 interacts with four distinct receptors in the body and exhibits many other physiological effects including muscle contraction and relaxation, hypotension and various gastro-intestinal effects that render its use for bone impractical [28].

Bone targeting prodrugs of prostaglandins E_2 and E_1

Some reports have described targeting PGE_1 to bone using an fluorescein isothiocyanate (FITC)-labelled hydroxymethylpolyacrylamide copolymer (P)–Asp8 conjugate (P-Asp8-FITC) [6] where the PGE_1 was coupled as an ester to the polymer and the fluorescein was used to gain a measure of bone attachment. Polyaspartate is known to bind to bone mineral due to its polycarboxylate structure. The polypeptide was a potential substrate for cathepsin K (the major protease secreted by bone resorbing osteoclast cells) and cleavage of the polymer could facilitate liberation of PGE_1 especially in areas of high bone turnover. Those authors found that a single injection of P-Asp8-FITC-PGE_1 in rats resulted in enhanced bone formation (relative to vehicle or P-Asp8-FITC) measured 4 weeks later but absolute amounts of polymer that reached the bone and of PGE_1 that was delivered were not defined [6].

In another study, several bone-targeting prodrugs of PGE_2 were prepared with the intent of devising a dual action prodrug that would release both PGE_2 as an anabolic agent and an antiresorptive bisphosphonate [29] (Figure 1). One conjugate (**1**) was prepared where alendronic acid was coupled to PGE_2 through an amide bond and this conjugate was radiolabelled with ^3H on PGE_2 and ^{14}C on alendronate to allow tracking *in vivo*. **1** was dosed to rats and found to be well taken up by bones (about 12–15% of administered dose), but tracking of the radiolabels over 4 weeks showed that the tritium (representing PGE_2) was not released and both labels remained unchanged, presumably due to the stability of the amide bond *in vivo*. In the same study a second conjugate (**2**) was prepared where PGE_2 was conjugated with a bisphosphonate via an ester bond at the 15-hydroxy group on PGE_2. Compound **2** was found to be quite stable in blood and a radiolabelled version (synthesized from tritiated PGE_2) was shown to be taken up into bones (about 3–3.5% of administered dose) and then to release the ^3H label with a half-time of about 7 days. Whereas PGE_2 was dosed intraperitoneally at 6 mg/kg (its maximum tolerated dose), the conjugate **2** could be dosed intravenously up to 100 mg/kg without overt side effects. Dosing **2** intravenously at 10 and 100 mg/kg once weekly for 28 days resulted in a dramatic anabolic effect and was shown to be more effective as a bone growth stimulant than PGE_2 dosed daily at its maximum tolerated dose. A mixture of PGE_2 and **3** (5 mg/kg each) was dosed once weekly to show the benefit of conjugation and showed no significant anabolic or antiresorptive effects at those doses.

Unfortunately, while prodrug **2** was a potent and well tolerated anabolic agent it did not demonstrate antiresorptive activity and was difficult to prepare on larger scale due to the sensitivity of PGE_2 (a tendency for β-elimination of the 11-hydroxyl group under acidic or basic conditions and facile isomerization of the 5,6 double bond).

Subsequent to these studies, Machwate *et al.* [30] demonstrated that PGE_2 exerted its anabolic effect in bone through agonism at the EP4 receptor subtype and a number of very potent and highly selective EP4 receptor agonists were subsequently identified [28]. One of these compounds (**4**) (Figure 2) was much more stable than PGE_2 both chemically and metabolically, was found to be orally absorbed [31], and was tested in rats for bone effects. Compound **4** dosed orally at 0.5 mg/kg for 28 days demonstrated anabolic effects similar to PGE_2 dosed at 3 mg/kg (R Young and G Rodan, unpublished data). Unfortunately, EP4 selective agonists were found to retain some of the systemic and GI side effects of PGE_2 and thus far have not been developed further for treatment of osteoporosis.

Bone targeting bisphosphonate conjugates delivering EP4 receptor agonists

The option of preparing a bone-targeting bisphosphonate conjugate as a prodrug to deliver an EP4 agonist to bones was revisited in a recent series of studies. In particular, the