NOVEL VITAMIN B_6 DERIVATIVE OF ALENDRONATE RAPIDLY ABSORBED FOLLOWING ORAL ADMINISTRATION

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ABSTRACT

Poor pharmacokinetics and upper GI irritation associated with oral bisphosphonate therapies are significant drawbacks to otherwise effective treatments for osteoporosis and other metabolic bone diseases. To address these problems we have attempted to provide a means for facilitated uptake of bisphosphonate via covalent attachment of vitamin B_6. The chemistry of attachment is amenable to any bisphosphonate containing a free primary amino group, such as pamidronate and alendronate. We used ^{14}C-radiolabeled alendronate and labeled alendronate-vitamin B_6 conjugate MBC-31 for pharmacokinetic studies. In rats dosed orally, the C_{max} of MBC-31 (~6.3 μg/ml) was double that of alendronate, and the T_{max} of MBC-31 was ~5 minutes as compared to ~30 minutes for alendronate. While the oral bioavailability (~0.7%) was essentially unchanged by conjugation, the clearance of MBC-31 from plasma was nearly doubled and may suggest an increase in tissue absorption. Furthermore, the distribution of alendronate and MBC-31 to bone appeared to be equivalent. Recent data from an estrogen deficiency-induced bone loss ovariectomized rat model of osteoporosis showed an MBC-31 induced reduction of serum and urine biomarkers of bone turnover and an improvement in total bone density.

INTRODUCTION

While millions of post menopausal women are currently taking bisphosphonates for the prevention and treatment of osteoporosis, less than half adhere to therapy regimens [1, 2]. Non-compliance is largely attributed to the following dosing requirements, *i.* no prior food, *ii.* the pill is taken with water, *iii.* no food or drink for at least 30 minutes post-dosing and *iv.* the person must remain in an upright position for at least 30 minutes.

We report our attempt to overcome these problems by improving the pharmacokinetic properties of alendronate via conjugating with vitamin B_6. It has been shown, that vitamin B_6 absorption from the food undergoes a specific transporter-facilitated mechanism [3, 4, 5].

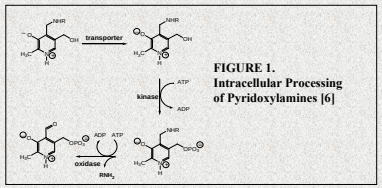


FIGURE 1. Intracellular Processing of Pyridoxylamines [6]

Furthermore, Zhang and McCormick [6] (Fig.1) have shown that many amines bearing a pyridoxyl group on the nitrogen enter cells by a transport system for the various forms of vitamin B_6 and, once inside, they are phosphorylated on O-5' by pyridoxal kinase (EC 2.7.1.35) and then oxidized by pyridoxaminephosphate oxidase (EC 1.4.3.5) to release pyrdoxal phosphate and the amine. Among the amines shown to behave in this way was the amino acid β-alanine. This leads us to believe that there is a high likelihood that metabolic degradation of B_6-conjugates will result in the release of free bisphosphonate and pyridoxal 5'-phosphate. The broader applicability of N-pyridoxylamines for delivery of amines can be appreciated when it is realized that almost all facultative and aerobic cells have both pyridoxal kinase and pyridoxamine-phosphate oxidase [5]. However, it is also possible that the intact compound will inhibit the same target enzyme (FPPS, Farnesyl diphosphate synthase, EC 2.5.1.10) of aminobisphosphonates demonstrating antiresorptive and/or anti-cancer activity.

METHODS

Synthesis

The conjugate of vitamin B_6 and alendronate was prepared in high yield, using standard conditions of reductive amination [7] as shown in Figure 2. Thus pyridoxal 1 was reacted with alendronate to produce the corresponding Schiff base 2. This intermediate was reduced *in situ* by sodium borohydride to give the desired conjugate 3 after ion-exchange purification and isolation. The conjugate structure was confirmed by NMR- and mass-spectroscopy. The [^{14}C] version of compound 3 was prepared using the same procedure starting from 1-[^{14}C]-alendronate and pyridoxal.

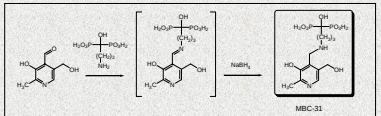
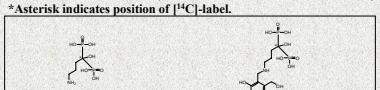


FIGURE 2. Synthesis of MBC-31

Pharmacokinetic Studies

MBC-31 and Alendronate were radiolabeled with [^{14}C] at the bisphosphonate geminal carbon (Figure 3) and had identical specific activities of 54 mCi/mmol (Moravek Biochemicals, Inc. Brea, CA). *Asterisk indicates position of [^{14}C]-label.



1-[^{14}C]-Alendronate [^{14}C]-MBC-31

FIGURE 3. Structures of radiolabeled alendronate and MBC-31

Tissue Distribution - Plasma, Kidney, Femur and Bone Marrow
Male Sprague-Dawley rats Crl:CD(SD)BR rats 8-12 weeks old
Femoral vein cannulated - Groups of 3/time point
8 hour fast prior to dosing
Intravenous administration
Alendronate Dose 0.4 mg/kg = 100 μCi/kg
MBC-31 Dose 0.76 mg/kg = 100 μCi/kg
Tissues collected at 30, 60 and 300 minutes
Plasma – heparin, cardiac puncture following CO_2 anesthesia
The activity per gram of tissue was measured by scintillation counting

Oral Bioavailability

Male Sprague-Dawley rats Crl:CD(SD)BR rats 8-12 weeks old
Three animals per group - Femoral vein cannulated
IV-Alendronate; PO-Alendronate; IV-MBC-31; PO-MBC-31
8 hour fast prior to dosing
Administration - Single bolus intravenous or oral gavage
Alendronate Dose 0.4 mg/kg = 100 μCi/kg
MBC-31 Dose 0.67 mg/kg = 100 μCi/kg
Blood was collected at 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12 and 24 hours; total urine and feces were also collected
The activity per gram of tissue was measured by scintillation counting

Efficacy Studies

Estrogen Deficiency – Induced Bone Loss, Ovariectomized Rat - model of osteoporosis
Virgin female, 3 month old Sprague-Dawley rats
Ovariectomy (OVx) or sham surgery
Eight animals per group
OVx-PBS, OVx-Alendronate, OVx-MBC-31 and Sham-PBS
Administration - Daily oral gavage for six weeks
late afternoon to approximate predose fast
Alendronate Dose 4.0 mg/kg/day
MBC-31 Dose 7.6 mg/kg/day
Endpoints:
Week 2
Serum CTX (C-telopeptide) via Immuno-assay
Serum OC (Osteocalcin) via Immuno-assay
Urinary DPD (deoxypyridilinoline) via Immuno-assay
Urinary CR (creatinine) – to normalize DPD – colorimetric assay
Week 6
Serum and Urine markers of bone turnover as above,
pQCT (quantitative computer tomography) of distal and midshaft femur, total bone mineral content, total bone area, total bone mineral density, trabecular bone mineral content, trabecular bone area, trabecular bone mineral density – for the midshaft cortical bone mineral content, cortical bone area, cortical bone mineral density, periosteal perimeter and endosteal perimeter was analyzed

RESULTS

Pharmacokinetic Studies

Biodistribution studies in rats with internally radiolabeled compound

Pharmacokinetic studies using radiolabeled MBC-31 and alendronate (Figure 3) were conducted in male Sprague-Dawley rats. Animals in groups of three were intravenously (IV) injected with ~1 mg/kg of radiolabeled MBC-31 or the control compound alendronate. Femur, bone marrow, plasma and kidney associated radioactivity was measured 30, 60 and 300 minutes post dosing. The resulting distribution is shown in Figure 4. The data is plotted on the same scale (μg compound / μg tissue) to allow comparison across tissues. Alendronate behaved as expected with a rapid clearance from plasma through the kidney and the majority of compound delivered to the bone [8]. No significant change in the amount of alendronate on the bone over the 5 hours of this experiment is consistent with rapid trafficking and binding to the bone with little or no dissociation. The same profile was observed for MBC-31, indicating that the vitamin B_6 moiety is not interfering with tissue distribution, once the compound is in the bloodstream.

Oral bioavailability and excretion studies in rats

Plasma levels of MBC-31 and alendronate were measured over a 24 hour time course after IV or PO (oral gavage) administration to assess the ability of MBC-31 to alter the absorption profile of alendronate. Total urinary and fecal excreta were collected 24 hours post dosing. The AUCs for MBC-31 and alendronate are ~ 0.7% indicating no significant change in the total amount absorbed. Strikingly, the plasma concentrations of MBC-31 just 5 minutes after PO dosing were the highest levels observed and were greater than 5-fold that as compared to alendronate (Figure 5). The difference is further reflected in the values of T_{max} for MBC-31 and alendronate as 5 and 30 minutes respectively. It appears MBC-31, as compared to alendronate, is both absorbed and cleared more rapidly from the bloodstream, and therefore suggests a different mechanism of uptake is involved.

Both alendronate and MBC-31 demonstrated the same routes of elimination as assessed by urinary and fecal content. When administered intravenously the compounds were renally eliminated, while PO resulted in eliminated compound found primarily in the fecal matter. This is consistent with the elimination profiles for bisphosphonates with low oral bioavailability (8).

In summary, overall bioavailability was not impacted by conjugation, while a significant improvement in the rate of absorption from the gut was observed. Ideally, this would translate into a reduction or complete elimination of the time required after dosing and prior to food and drink consumption.

In vivo Efficacy Experiments

Estrogen deficiency-induced bone loss - Ovariectomized (OVx) rat model of osteoporosis

We assessed the biological activity of an alendronate-vitamin B_6 conjugate, MBC-31, in a rat model of osteoporosis. Three month old virgin female Sprague-Dawley rats were divided into four groups of eight. The first group received a sham operation with the initiation of daily oral dosing of vehicle (PBS). The second group was ovariectomized and dosed daily with vehicle and used as an osteoporotic control. The third group was ovariectomized and dosed daily with 4 mg/kg/day alendronate as the positive control, and the fourth group was ovariectomized and dosed daily with an equimolar amount of MBC-31. After six weeks of dosing, serum and urine biomarkers of bone turnover, as well as bone density were measured.

Figure 6-A demonstrates that the reduction of serum levels of C-telopeptide (CTX) caused by MBC-31 was even greater than that of the alendronate. Since CTX is a biomarker of osteoclast activity we conclude our compound not only traffics to the bone site and is absorbed by osteoclasts, but that it has similar activity as its parent compound alendronate. Deoxypyridinoline (DPD) is a specific metabolite of bone resorption [9]. The amount of DPD in urine, normalized to the amount of creatinine (Cr), is a very specific biomarker of the osteoclast activity [9]. Figure 6-B shows that in animals dosed with MBC-31 the urine levels of DPD/Cr are reduced by 50% as compared to the OVx control, which is a direct indication of MBC-31 antiresorptive activity. Figure 7 demonstrates MBC-31 dosing has restored 40% of the bone density loss caused by ovariectomy. Thus, our preliminary efficacy data warrants further development of vitamin B_6–bisphosphonate conjugates for the treatment of metabolic bone diseases.

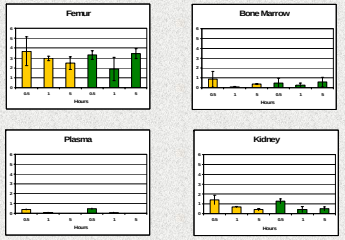


FIGURE 4. Tissue distribution after a single IV bolus dose of MBC-31 (green) and alendronate (gold). The y-axis is standardized for all tissues and presented as μg compound / μg tissue. Error bars represent the standard deviation.

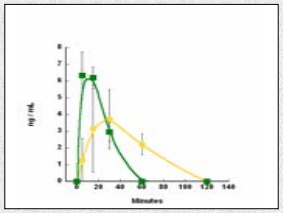


FIGURE 5. Plasma concentrations after a single PO bolus dose of MBC-31 (green) and alendronate (gold). Error bars represent the standard error of the means.

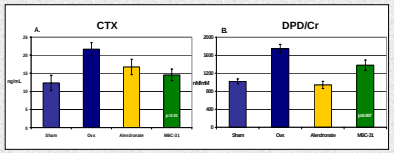


FIGURE 6. The effects of MBC-31 on serum and urine biomarkers of bone resorption A. Serum levels of CTX after six weeks of dosing. B. Urinary levels of DPD/Cr after six weeks of dosing. Error bars represent the standard error of the mean and the p-values are relative to the untreated OVx control group.

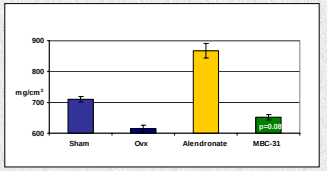


FIGURE 7. The effects of MBC-31 on total bone density. The total bone density values were determined by quantitative computer tomography of 0.5 mm sections of the distal femur. Error bars represent the standard error of the mean and the p-values are relative to the untreated OVx control group.

CONCLUSIONS

We developed a novel vitaminB_6-bisphosphonate conjugate with improved absorption as compared to alendronate.

MBC-31 demonstrated a significantly reduced T_{max} compared to alendronate.

Equimolar dosing of MBC-31 and alendronate provided similar AUC values.

MBC-31 traffics to the bone at levels similar to alendronate.

Distribution to kidney, urine, feces, bone marrow and blood are similar for both compounds.

In OVx animals MBC-31 has demonstrated efficacy based on:
Reduced serum CTX
Reduced urinary DPD/Cr
Increased total bone mineral

The current data does not indicate the extent to which alendronate is released from MBC-31; however, it appears release is not so fast as to enable identical biological outcomes.

Overall, our data supports the concept of using vitamin B_6 conjugation to improve the absorption of bisphosphonates, which will lead to an improved oral dosing regimen for the treatment of metabolic bone diseases

ACKNOWLEDGEMENTS

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