Bisphosphonates and Alendronate

Summary

Bisphosphonates are a class of drugs used in the management of disorders of calcium and bone metabolism. Treatment with bisphosphonates causes early reduction in bone resorption and then a later reduction in bone formation. They bind strongly to bone mineral, and inhibit the bone resorption and crystal dissolution.

Alendronate, like other bisphosphonates, is a bone resorption inhibitor being used in the prevention and treatment of bone diseases. It is used in the prevention and treatment of post-menopausal osteoporosis, osteoporosis in men, corticosteroid-induced osteoporosis, Paget's disease, primary hyperparathyroidism, malign hypercalcemia and metastatic bone diseases. The bioavailability of alendronate is low. It is primarily absorbed across the gastrointestinal tract paracellularly because of the polar and charged structure at physiological pH. It is better absorbed from the segments of the gastrointestinal tract with larger surface areas. Following the administration of intravenous $^{14}$C alendronate, the drug distributes in the calcified and noncalcified tissues. Albumin is the predominant protein that binds alendronate. It appears not to be metabolized in mammals. The renal route is the only means for its elimination. Terminal elimination half-life in women with post-menopausal osteoporosis is 10 years, while it is 200 days in rats and 1000 days in dogs.

Alendronate is generally well-tolerated after short- or long-term usage, but adverse effects like esophagitis and gastric damage have also been reported. It can behave like a topical irritant and can affect the gastric epithelium directly or indirectly by increasing esophageal irritation that was present before. It can increase esophageal abnormalities caused from low pH.

The direct chromatographic analysis of trihydrate of alendronate sodium is complicated due to the lack of a suitable UV chromophore for conventional high-performance liquid chromatographic analysis and insufficient volatility for gas chromatographic analysis.

**Key Words:** Bisphosphonates, alendronate, therapeutic use, pharmacokinetics, adverse effects, analysis.

Received: 31.01.2007
Revised: 11.06.2007
Accepted: 26.06.2007

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Bisphosfonatlar ve Alendronat

Ozet


Alendronat trihidratının doğrudan kromatografik analizi, yüksek basınclı son kromatografideki analizi için uygun UV kromofor grup taşmadığında, gaz kromatografik analizi için ise uygulunun yetersiz olmasından dolayı karşiştıktır.

**Anahtar Kelimeler:** Bisphosfonatlar, alendronat, terapeutik kullanım, farmakokinetik, yan etkiler, analiz.
INTRODUCTION

Bisphosphonates are a class of drugs used in the management of disorders of calcium and bone metabolism. They are analogs of inorganic pyrophosphate. They contain carbon atom rather than oxygen in pyrophosphate, which allows a great number of possible variations (Fig. 1).

Inorganic pyrophosphate can prevent calcification by binding hydroxyapatite (bone mineral) crystals. Orally used pyrophosphate is inactivated in the gastrointestinal tract by mucosal brush border phosphatases. Bisphosphonates bind strongly to hydroxyapatite crystals. They had become resistant to enzymatic hydrolysis due to the carbon atom in their structure.

The activity of bisphosphonates differs from one to another due to the length and substitution of the aliphatic carbon atom. Bisphosphonates have many derivatives (Table 1).

The first therapeutically used bisphosphonate is etidronate.

Bisphosphonates prevent the osteoclast-mediated bone resorption. The potency of inhibiting bone resorption varies between different bisphosphonates by up to 5000-fold. Their antiresorptive activity is, in increasing order, as follows: etidronate < tiludronate < clodronate < pamidronate < alendronate < risedronate.

In this review, a short discussion of the therapeutic applications, mechanism of action and structure-activity relationship of bisphosphonates will be reviewed and detailed information about alendronate will be discussed.

Therapeutic applications of bisphosphonates

Bisphosphonates increase the calcium balance and mineral density of bone. They are used in Paget’s disease, hypercalcemia, tumor-induced hypercalcemia, postmenopausal and corticosteroid-induced osteoporosis, primary and secondary hyperparathyroidism and bone metastases. After the therapy in malignant bone problems with bisphosphonates, the bone complications of malignity are decreased. Some of the bisphosphonates have direct antitumoral effects. The bisphosphonates available for oncological use are, in order of increasing potency: etidronate, clodronate, pamidronate, alendronate, ibandronate and zoledronate.

They can also be used as a bone scanner. This is based on the properties of high affinity for bone mineral and the ability to bind Tc-99m isotope. In this manner, the diagnosis of bone metastases and other bone lesions is possible. Bisphosphonates, particularly pamidronate and partly clodronate, have an analgesic effect in patients with symptomatic osteolytic metastases and thus these can be used in association with conventional oncological treatments for the management of metastatic bone diseases.

Etidronate has been reported to inhibit the thickness of the carotid artery wall in men. Bisphosphonates

![Image](image_url)
are also under investigation for the treatment of fibrous dysplasia and osteogenesis imperfecta.\(^3\)

**Structure-activity relationship**

The structure, functional groups and structure-activity relationship of bisphosphonates are shown in Figure 2. The carbon atom bridges the two phosphate residues, which renders bisphosphonates chemically stable and able to withstand incubation in acids or with hydrolytic enzymes. The P-C-P moiety is responsible for the strong affinity of the bisphosphonates for the skeleton and allows for a number of variations in structure based on substitution in the R1 and R2 positions on the carbon atom.\(^2\)

Bisphosphonates containing a primary nitrogen atom in an alkyl chain (as in pamidronate and alendronate) were found to be 10-100-fold more potent than etidronate and clodronate. The most potent antiresorptive bisphosphonates were those containing a nitrogen atom within a heterocyclic ring (as in risedronate and zoledronate). For maximal potency, the nitrogen atom in the R2 side chain must be a critical distance away from the P-C-P group, and in a specific spatial configuration.\(^2\)

Both phosphate groups are required for the drugs to be pharmacologically active. Alterations to one or both phosphate groups reduce the affinity for bone mineral.\(^2\) Replacement of one of the phosphate hydroxyl groups with a methyl group markedly reduces both bone affinity and antiresorptive potency. Methylation of both phosphate groups leads to loss of bone affinity and loss of antiresorptive activity in vivo.\(^3\)

**Mechanisms of action**

Bisphosphonates form polymeric aggregates with calcium in biological systems. They show a high affinity for mineralized bone matrix, which explains their pharmacological effects in the target tissue.\(^3\)

Treatment with bisphosphonates causes early reduction in bone resorption followed by a later reduction in bone formation. They bind strongly to bone mineral and inhibit the bone resorption and crystal dissolution. Bisphosphonates are selectively internalized by osteoclasts rather than other cell types because of their accumulation in bone and the endocytic activity of osteoclasts. The subcellular space beneath the osteoclasts becomes acidic during the bone resorption. This acidic pH causes the dissolution of bone mineral. Osteoclasts are exposed to bisphosphonates, which are released from the bone mineral in the low pH, because bisphosphonates adsorb to the spaces where bone resorption occurs.\(^3\)

Bisphosphonates affect osteoclast-mediated bone resorption in such ways as their effects on osteoclast differentiation and resorptive activity.\(^3\) Once internalized in osteoclasts, they disrupt the cellular metabolism and induce apoptosis.\(^2\) The actions of bisphosphonates are shown in Figure 3.

The effects of bisphosphonates on bone resorption can be considered at three levels: a) Tissue level: The action of all active bisphosphonates appears to be similar - a reduction in bone turnover. This is evidenced by a decrease in bone resorption and bone
formation\textsuperscript{21}, b) Cellular level: They inhibit the osteoclast bone resorption directly and/or indirectly and inhibit the osteoclast-mediated cytokine formation directly\textsuperscript{7}, c) Molecular level: Tiludronate inhibits vacuolar ATPase; several bisphosphonates inhibit squalene synthase, and all bisphosphonates tested inhibit protein tyrosine phosphatase\textsuperscript{21}.

Bisphosphonates can inhibit the bone resorption by preventing osteoclast formation\textsuperscript{29}. Sahni et al.\textsuperscript{37} has promoted that the inhibitory effect on osteoclasts are via osteoblasts. This situation occurs when osteoblasts are stimulated to form osteoclast-inhibitory factor\textsuperscript{37-39}. It is still unknown whether the direct effect on osteoclasts or indirect effect via osteoblasts is important in vivo\textsuperscript{40}.

\textbf{ALENDRONATE}

Structure and properties

The chemical structure and the properties of alendronate sodium trihydrate are shown in Figure 4 and Table 2, respectively.

![Figure 4. The chemical structure of alendronate sodium trihydrate\textsuperscript{41}.

| Table 2. Properties of alendronate

| Empiric formula | C\textsubscript{4}H\textsubscript{12}N\textsubscript{a}NaO\textsubscript{7}P\textsubscript{2}.3H\textsubscript{2}O \textsuperscript{42} |
| Melting point | 260\textdegree C \textsuperscript{43} |
| Soluble in | Soluble in water, very slightly soluble in alcohol and practically insoluble in chloroform\textsuperscript{44} |
| Solubility | Different crystal forms have different solubility\textsuperscript{9} |
| Octanol/buffer partition coefficient | 0.0017 and independent of the pH values between 2-11\textsuperscript{46} |
| pKa | 0.8, 2.2, 6.3, 10.9 and 12.2\textsuperscript{45} |
| Ionization | Wholly ionized and charged negatively in the physiological pH of the small intestine\textsuperscript{45} |

\textbf{Therapeutic use}

Alendronate, like other bisphosphonates, is a bone resorption inhibitor being used in the prevention and treatment of bone diseases\textsuperscript{13}. It is used in the prevention and treatment of post-menopausal osteoporosis\textsuperscript{46,47}, osteoporosis in men\textsuperscript{48}, corticosteroid-induced osteoporosis\textsuperscript{49}, Paget’s disease\textsuperscript{50}, primary hyperparathyroidism, malignant hypercalcemia and metastatic bone diseases\textsuperscript{51}. Clinical effect of alendronate is the increase in bone mineral density, bone strength and decrease in the risk of fracture\textsuperscript{47}.

\textbf{Mechanism of action}

Alendronate is a nitrogen-containing bisphosphonate\textsuperscript{52}. It binds to bone due to the high affinity for calcium ions\textsuperscript{34,48}. It binds to the spaces where bone turnover takes place\textsuperscript{35}, reduces the bone turnover, and decreases the risk of fracture by increasing the density, period of bone mineralization and bone strength\textsuperscript{48}.

The possible mechanisms of osteoclast-mediated bone resorption with alendronate are: a) Reduces osteoclast number and induces apoptosis\textsuperscript{29}, b) Disruption of the cytoskeleton or ruffled border of osteoclasts\textsuperscript{29}, c) Inhibition of the formation of osteoclasts\textsuperscript{53}, and d) Effects on osteoclast precursors\textsuperscript{37}.

The metabolic effects of alendronate are: a) Inhibition of the steps in the mevalonate pathway\textsuperscript{11} and b) Inhibition of protein-tyrosine phosphatase\textsuperscript{54}.

The latest studies have focused on the fact that the mevalonate pathway and protein prenylation are inhibited by alendronate. As shown in Figure 5, alen-
dronate is a specific and potent inhibitor of farnesyl diphasate synthase in the mevalonate pathway. Higher molecular concentrations do not inhibit other enzymes in the pathway. Farnesyl diphasate synthase and geranylgeranyl diphasate are necessary for the prenylation of many proteins and these proteins are necessary for skeleton organization.

**Absorption**

The bioavailability of alendronate is low and the plasma concentrations following oral administration are under the limit of quantification, thus the limited information of the pharmacokinetic data was obtained from urine excretion data. The pharmacokinetics of alendronate in humans is generally concordant with animals. The average bioavailability is less than 1%. The oral bioavailability is 0.64% in women when 5-70 mg drug is taken after all night and two hours before breakfast. Bioavailability of 10 mg alendronate in men under the same conditions is 0.59%. Bioavailability is reduced when the drug is taken with divalent cations like calcium, or with beverages other than water with breakfast or during two hours after breakfast. The increase in gastric pH causes increases in bioavailability. The absorption and elimination are linear between the doses of 5-80 mg. Ionic conditions have little effect on absorption.

The oral bioavailability of alendronate in animals in the presence of food is also low. The fasting oral bioavailability of alendronate was estimated as 0.9%, 1.8% and 1.7% for rats, dogs and monkeys, respectively. Oral administration to rats in the presence of food decreases bioavailability 6- to 7-fold.

Alendronate is primarily absorbed across the gastrointestinal tract paracellularly because of the polar and charged structure at physiological pH. It is better absorbed from the segments of the gastrointestinal tract with larger surface areas (jejunum > duodenum > ileum).

**Distribution**

Following the administration of the intravenous 14C alendronate, the drug distributes in the calcified and noncalcified tissues. Once the drug binds to the surface of bone mineral, one-third of the bound drug slowly passes to the plasma and eliminates via kidneys. After the intravenous administration, 40% of the dose is found in the urine in 36 hours.
An intravenous dose of alendronate 1 mg/kg in rats is quickly and widely distributed throughout the body followed by redistribution to its ultimate site of sequestration (bone) or elimination. About 60% of the dose is present in noncalcified tissues at 5 min post-dose. This is reduced to about 5% by 1 h and about 1% at 6 to 24 h post-dose. A reciprocal pattern is evident in bone, where about 30% of the dose can be found 5 min after administration, reaching some 60 to 70% of dose by 1 h, and remaining constant for the next 71 h (Figure 7)\textsuperscript{58}.

![Figure 7. Distribution of alendronate to soft tissues and bone in rats (n=3 to 4) following administration of a single intravenous dose of 1 mg/kg.](image)

Alendronate is effective on bone surface and does not inhibit the resorption when internalized in bone matrix\textsuperscript{40}. The amount of alendronate in bone depends on the bone turnover rate. The duration in the skeleton increases and urine elimination decreases with increased turnover rate\textsuperscript{60}. The distribution of alendronate within bone is determined by blood flow\textsuperscript{58}. Binding to bone can be saturated\textsuperscript{35}. A larger proportion of dose is taken up by the trabecular bone\textsuperscript{58}. High doses of etidronate inhibit the uptake of alendronate to bone in rats\textsuperscript{40}.

The binding of alendronate with plasma proteins is dependent on species\textsuperscript{40}. The ratio of binding to proteins in human plasma is 78%\textsuperscript{42}. Albumin is the predominant protein that binds alendronate. The binding degree depends on pH and calcium. Over the concentration range of 0.1 to 0.5 mg/ml, alendronate is approximately 80, 73 and 70% protein bound in rats, dogs and monkeys, respectively\textsuperscript{40,50}.

**Metabolism**

Alendronate appears not to be metabolized in mammals\textsuperscript{19,40,58,61} and does not eliminate via bile\textsuperscript{19,61}.

**Elimination**

Renal route is the only means for the elimination of alendronate. The elimination via kidneys is dependent on the concentration\textsuperscript{40,62} and can be saturated\textsuperscript{40}.

Orally administrated alendronate is distributed and eliminated like parenterally administrated alendronate\textsuperscript{40}. Alendronate is eliminated renally via an active canal different from the filtration and classical acid-base canal\textsuperscript{19,61}. Only a negligible amount of the drug (<0.2%) is detected in feces after intravenous administration\textsuperscript{55}. The systemic clearance of alendronate is more than 11.94 L/h and the steady state volume of distribution is more than 28 L. Renal clearance is 4.26 L/h\textsuperscript{61}.

The terminal half-life of alendronate is related to the rate of bone turnover\textsuperscript{58}. The one-third of absorbed alendronate remains in bone for a long time\textsuperscript{48}. Once bound to bone, the first elimination half-life on bone surface is a few weeks\textsuperscript{60}. Terminal elimination half-life in women with post-menopausal osteoporosis is 10 years\textsuperscript{19,60,61}, while it is 200 days in rats and 1000 days in dogs\textsuperscript{58,59}.

**Adverse events**

Alendronate is generally well-tolerated after short- or long-term usage\textsuperscript{48}, but adverse effects like esophagitis and gastric damage have also been reported\textsuperscript{63,64}. Alendronate has been shown to be toxic in the upper gastrointestinal tract with many endoscopic studies\textsuperscript{65,66}. Alendronate has an acute mucosal damage rate comparable with nonsteroidal antiinflammatory drugs\textsuperscript{65}.

Abdominal pain, nausea, dyspepsia, constipation and diarrhea were reported in 3 to 7% of patients receiving alendronate in two large three-year studies\textsuperscript{67} (Fig. 8).
10 mg alendronate for one month causes gastrointestinal adverse effects more than pamidronate and etidronate. It causes adverse effects similar to or more than risedronate. The safety profile of alendronate is not dependent on age, species or renal function.

Post-marketing data obtained with the use of alendronate is shown in Table 3.

Table 3. Patients and adverse esophageal effects associated with the use of alendronate, according to post-marketing data

<table>
<thead>
<tr>
<th>Adverse effect</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any effect †</td>
<td>199</td>
</tr>
<tr>
<td>Serious or severe effect †</td>
<td>51</td>
</tr>
<tr>
<td>Esophageal ulcer</td>
<td>22</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>21</td>
</tr>
<tr>
<td>Erosive esophagitis</td>
<td>13</td>
</tr>
<tr>
<td>Esophagalgia or odynophagia ‡</td>
<td>12</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>11</td>
</tr>
<tr>
<td>Acid regurgitation or dyspepsia ‡</td>
<td>5</td>
</tr>
<tr>
<td>Reflux esophagitis</td>
<td>4</td>
</tr>
<tr>
<td>Hematemesis or esophageal hemorrhage ‡</td>
<td>2</td>
</tr>
<tr>
<td>Esophageal stricture</td>
<td>2</td>
</tr>
</tbody>
</table>

* As of March 5, 1996, an estimated 475,000 patients worldwide had received prescriptions for alendronate.
† Patients with more than one type of event were counted only once.
‡ Patients with both events counted only once.

Alendronate can behave like a topical irritant and can affect the gastric epithelium directly or by increasing the esophageal irritation that was present before. It can increase the esophageal abnormalities caused from low pH.

Generally, people with gastrointestinal adverse effects do not comply with the instructions or they may have esophageal disorders that have not been diagnosed before. To reduce the adverse effects and increase the bioavailability, alendronate should be taken with water in the morning, 30 min before breakfast, beverages or before another drug. Patients should not lie down for at least 30 min or till the first food of the day. A decrease has been observed in esophagitis and esophageal ulceration numbers when patients comply with these instructions. Drug should be given cautiously in patients who have swallowing problems or abnormal esophageal dynamism, and should be cut off when esophageal symptoms are observed.

Analysis methods

The direct chromatographic analysis of trihydrate alendronate sodium is complicated due to the lack of a suitable UV chromophore for conventional high-performance liquid chromatographic (HPLC) analysis and insufficient volatility for gas chromatographic analysis. Alendronate is derivatized with some reagents from the amino group and thus has chromatographic and UV properties that enable facile assay by HPLC. Methods for its determination in pharmaceutical formulations are based on liquid chromatography-mass spectrometry, ion chromatography with indirect UV detection, or conductivity detection, capillary electrophoresis, inductively coupled plasma, ion chromatography with refractive index detection, spectrophotometric determination via complex formation with Fe (III) ions, HPLC analysis using postcolumn derivatization, and fluorescence detection.

Quantification of alendronate in biological fluids was performed by automated precolumn derivatization and HPLC with fluorescence and electrochemical detection, automated precolumn derivatization with 2,3-naphthalene dicarboxyaldehyde and HPLC with fluorescence detection, derivatization with o-phthaldehyde and HPLC with fluorescence detection, and derivatization with 9-fluorenylmethyl and HPLC with fluorescence detection.

Formation of a chromophoric complex between alen-
dronate and copper (II) ions utilization of copper (II) phosphate for the anodic atripping voltametric assay and ion chromatography-inductively coupled plasma mass spectrometry are methods for alternative analysis.

In our study (data not shown), we used the derivatization process with 9-fluorenylethyl chloroformate as described by De Marco et al.43 and obtained a suitable UV chromophore for conventional HPLC analysis.

Commercially available products

Commercially available products of alendronate in the Turkish Drug Industry are all in tablet forms. Tablet formulation can cause adverse effects as described above. Thus, in our study, we aimed to prepare a drug carrier system which will hinder the adverse effects of alendronate and can also improve its oral bioavailability. We decided to prepare a microemulsion formulation of alendronate consisting of Captex-200®, Phospholipon 90 NG®, propylene glycol and bidistilled water as oil, surfactant, co-surfactant and water phases, respectively. The physical characterization of the formulation (physical appearance, viscosity, refractive index, conductivity, density and turbidity) was investigated at 4°C and 25°C over six months while droplet size was investigated for three months. None of the parameters except refractive index changed significantly during the determined periods. The mean size of the microemulsion droplets was approximately 1.21-1.36 nm and remained constant throughout the experiment. As a consequence, we succeeded in preparing a stable microemulsion formulation of alendronate.

CONCLUSION

Alendronate is a member of the bisphosphonates used in bone diseases. The mechanism of action of alendronate is not clear, but the latest studies have focused on the mevalonate pathway. The bioavailability of alendronate is low. It is commercially prepared as a tablet formulation. However, side effects like esophagitis associated with the use of alendronate have been reported. Hence, future researches should focus on formulation studies of alendronate that will increase its bioavailability and decrease the side effects.

REFERENCES


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