PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

In Vitro Determination of the Release of Alendronic Acid from Alendronate Tablets of Different Brands during Deglutition

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ABSTRACT: Alendronic acid, a frequently applied compound for the treatment of different forms of diseases of bone metabolism, is a strong acid with a high solubility in water. In connection with the oral administration this exhibits a potential health risk for the upper gastrointestinal tract. The *in vitro* release of tablets containing alendronic acid of different brands (Stada®, ratiopharm®, interpharm®, Fosamax®) was determined by dissolution tests for the time period required for oral intake. The effect of rotation speed, temperature, and solvent volume on the release rate of alendronic acid was determined for the used dissolution apparatus. Analysis of alendronic acid was performed by a validated HPLC method. The highest rate of release was found for the original brand. The dissolution rate of the generic formulations was significantly lower in the early stage of dissolution. Over the complete range of dissolution, more than 85% of the claimed amount was dissolved within 4 min. Dissolution profiles were compared by calculation of the similarity factor f_2 showing equal products with the exception of one generic product, whose dissolution rate was lower. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:3575–3581, 2009

Keywords: alendronic acid; dissolution rate; deglutition; esophagitis; HPLC; formulation; distribution; solubility

INTRODUCTION

Bisphosphonates are diphosphate analogs of pyrophosphate in which the bridging oxygen has been replaced by carbon. In addition, several compounds have an alkylamine side chain. Absorption from the intestine is poor (up to 10%) and about 20–50% of the absorbed dose is taken up by the skeleton.[1,2] They suppress osteoclastic-mediated bone resorption, which leads in the long term to the increase of bone mass. Because of their high potential for the management of various diseases of bone metabolism, they are applied extensively in the treatment of various forms of osteoporosis, malignant hypercalcemia, and Paget's disease of the bone.[3,4]

Alendronic acid (4-amino-1-hydroxybutyliden-diphosphonic acid), a frequently applied bisphosphonate, is a polyvalent strong acid with a high

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solubility in water.[5,6] It is administered orally as its monosodium salt in the form of tablets. When coming into contact with water, alendronic acid is immediately released into solution. As a consequence, the dissolution of the tablets starts already during deglutition and may induce esophagitis.[7,8] The drug should therefore be taken in the morning with a full glass of water and an upwards position and the patient should not lie down or intake food in the following 30 min. The fast release of alendronic acid exhibits a potential health risk, which should be kept as low as possible.

At present pharmaceutical formulations containing alendronic acid are available from several producers, which may differ in their quality towards the original product.

Disintegration tests, which measure the release of fines into solution, were performed on several alendronate formulations. Higher as well as lower disintegration rates in relation to the original brand were reported.[9,10] The actual amount of pharmaceutical agent released into solution can be determined by dissolution tests. The rate and time required for the complete dissolution of the formulations are easy accessible by these methods. Measurements concerning the early state of dissolution, which covers the time period of deglutition, were not carried out so far. This study examines, how much alendronic acid can be expected to be released into solution during the time period required for oral intake of alendronate tablets and evaluates the differences between the original product and generic formulations in that respect. This was accomplished by *in vitro* dissolution testing measuring the rate of release in the first 20 s of dissolution. To evaluate the difference of the tested formulations, additional dissolution tests comprising the complete range of dissolution were carried out.

MATERIALS

Analytical grade potassium dihydrogen phosphate, disodium hydrogen phosphate, phosphoric acid, sodium hydroxide, potassium hydroxide, glacial acetic acid, and trisodium citrate monohydrate of analytical grade were purchased from VWR (Darmstadt, Germany) and 9-fluorenylmethylchloroformate (FMOC) from Fluka (Taufkirchen, Germany). Acetonitril and methanol were of chromatographic grade and from VWR. The analytical standards of sodium alendronate and disodium pamidronate were from Sigma (St. Louis, MO).

For analysis were used the generic formulations Alendrostad (Stada, Bad Vilbel, Germany), batch AR57A6, Alendronsaeure ratiopharm® (ratiopharm, Ulm, Germany), batch F31747, Alendronsaeure (interpharm, Vienna, Austria), batch 615485, and the original formulation Fosamax (Merck&Co., Inc., Whitehouse Station, NJ), batch R6302. The amount of alendronic acid in each tablet was 70 mg. In the text they are designated as product A, B, C, and D, respectively.

METHODS

Dissolution Tests

Dissolution tests were performed in a solution of phosphate buffer (pH 7.4) prepared by adding 250 mL of 0.2 M potassium dihydrogen phosphate to 393 mL of 0.1 M sodium hydroxide. One hundred milliliters of phosphate buffer was thermostatted in a 150 mL borosilicate glass vessel at 37°C by a Haake F3 Thermostat (Haake, Karlsruhe, Germany). A schematic outline of the experimental set-up is shown in Figure 1. The solution was agitated by a magnetic stirrer of type IKA RCT (IKA GmbH, Staufen, Germany) at 150 rpm with a stir bar of 2.0 cm length and 1.0 cm in diameter. The tablet was placed into a cage of coiled stainless steel. The cage with a length of 2.0 and 0.9 cm in diameter was closed on one side. Before starting temperature was controlled by a thermometer. At start time the cage was immersed into the buffer solution 1.5 cm below the liquid level and 1.5 cm from the center of the vessel. Samples of 0.5 mL were collected with a piston-stroke pipette after 5,10, 15, and 20 s. The immersion depth of the tip was 0. 4–0. 7 cm. The samples were transferred into 1.5 mL polypropylene reaction vials and stored at −18°C.

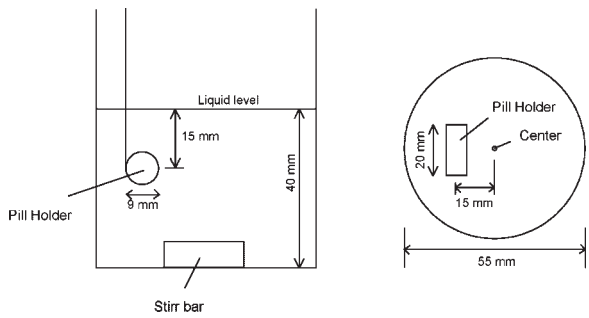

Figure 1. Schematic outline of the dissolution apparatus.

For the determination of the complete dissolution profile additional samples were collected in intervals of 1 min. A maximum difference of 0.1 pH units was observed between start and end of dissolution.

Derivatization and Chromatography

The quantitative determination of alendronic acid in solution is mostly performed by HPLC. The detection of alendronic acid as well as of most bisphosphonates is difficult because of the lack of chromophores. Therefore, detection methods such as indirect UV detection,[11] mass spectrometry,[12] or ICP[13] were applied. Another approach is the precolumn derivatization of the analytes to fluorescent derivates.[14–16] In this study HPLC with FMOC as agent for precolumn derivatization was applied for analysis.

Pamidronic acid differs from alendronic acid by an additional methylene group in the side chain and is therefore an ideal internal standard for the control of derivatization. The addition of pamidronic acid as internal standard enhanced the reproducibility of the method significantly.

In preliminary experiments variables, which effected reproducibility and accuracy of the method, were optimized.

For analysis samples of alendronic acid with concentrations below 4 μg/mL were used without further dilution. Samples of higher concentrations were diluted with phosphate puffer (pH 7.4). Derivatization was performed by mixing together 100 μL diluted sample solution, 100 μL internal standard solution of disodium pamidronate (4 μg/mL), 100 μL phosphate buffer for derivatization, and 100 μL FMOC-solution (4 mg/mL) in acetonitrile. The phosphate buffer for derivatization contained 23 g of potassium hydroxide and 27 g disodium hydrogen phosphate pentahydrate in 100 mL water. The FMOC solution was prepared daily.

The mixture was shaken and allowed to stand at ambient temperature for 1 h. The reaction was stopped by addition of 100 μL 2.5 M acetic acid.

Separation of the derivatized components was performed by HPLC on a Purospher Star octadecylsilica column of the dimension 250 mm × 4 mm i.d. at ambient temperature.

Eluent was delivered by a Merck/Hitachi L-6200 gradient pump and 20 μL of the derivatized samples was injected by a Merck/Hitachi L-4000 autosampler.

Eluent composition and elution programme of the ternary linear gradient are shown in Table 1. The analytes were detected by a Merck/Hitachi F-1080 Fluorescence detector at 260 nm excitation and measuring the emission at 310 nm.

The peaks of pamidronic acid and alendronic acid were baseline separated, not disturbed by other components and elute at 8.3 and 9.2 min, respectively.Data acquisition and quantitation were performed by chromatographic software.

RESULTS AND DISCUSSION

Chromatography

The effect of reaction time on the yield of derivatization was determined for alendronic acid and pamidronic acid. After 45 min the reaction yields of both compounds reached a maximum and did not increase for longer reaction times. Lower yields were found for reaction times below 30 min and above 8 h. Therefore, reaction time for the samples was set to 1 h.

Table 1. Eluents and Ternary Gradient Programme of the HPLC Method for Separation of Derivatized Alendronic Acid and Pamidronic Acid

Eluent A = 0.025 M sodium citrate adjusted to pH 4.9 with phosphoric acid
Eluent B = acetonitrile
Eluent C = methanol

Time (min)	% A	% B	% C	Flow (mL/min)	Comment
Linear gradient programme					
0	60	20	20	1.0	Start of analysis
9	50	20	30	1.0	
9.1	10	40	50	1.3	Cleaning of the column
14.0	10	40	50	1.3	
14.2	60	20	20	1.0	Reequilibration of the column
21.0	60	20	20	1.0	End of cycle

In acidic solution the reaction products were stable for at least 8 h, then decomposition was measureable. The reaction rate and reaction yield for pamidronic acid were lower than for alendronic acid.

The chromatographic system was calibrated daily with derivatized standard solutions of alendronic acid in phosphate buffer (pH 7.4) in the concentration range from 0.5 to 4.0 μg/mL ($n = 5$). Quantitative analyses were performed by calculation of the peak area ratio of alendronic acid to pamidronic acid. The individual calibration functions were highly linear with a correlation coefficient better than 0.9990. Validation parameters of the method are shown in Table 2. The overall precision and accuracy was better than 5% and 4%, respectively.

The relative high concentration of alendronic acid in the samples required operation of the fluorescence detector in the most insensitive mode. In the most sensitive detection mode, limit of quantitation is about two orders of magnitude lower.

Variables of Dissolution Testing

The main objectives of the system set-up were the establishment of a robust system for dissolution tests and the rapid distribution of the dissolved alendronic acid in solution. Variables, which effect the dissolution properties of the tablets in solution, are stirring rate, temperature, solvent pH, and position of the tablet in the dissolution apparatus.

Table 2. Validation Parameters of the Method for Analysis of Alendronic Acid in Aqueous Solution

	Alendronic Acid		
Within-day precision			
Concentration (μg/mL)	1.0	2.0	3.0
n	4	4	4
CV (%)	4.4	4.1	1.6
Between-day precision			
Concentration (μ/L)	1.0	2.0	3.0
n	7	7	7
CV (%)	4.9	4.8	3.9
Accuracy			
Within-day (%)	0.5	−2.2	−1.6
Between-day (%)	−0.5	−3.7	−3.6
Working range (μg/mL)	0.5–4.0		
Linearity (r^2)	>0.9990		
LOQ[a]	0.2 μg/mL		

n, number of determinations; CV, coefficient of variation.
[a]At lowest sensitivity of detection.

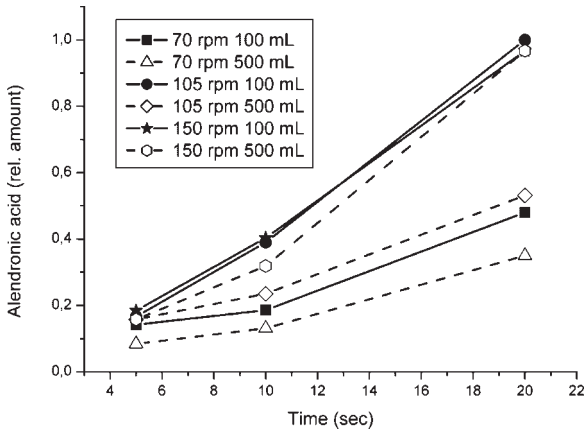

Figure 2. Effect of stirring rate and solvent volume on the release of alendronic acid at 37°C.

Tests investigating the effect of stirring rate and solvent volume on the release rate of alendronic acid were carried out in 100 and 500 mL phosphate buffer (pH 7.4) at 70, 105, and 150 rpm. The solvent was thermostatted to 37°C. The results are shown in Figure 2. At low rotation speed variations of the hydrodynamic conditions induced by fluctuations of the rotation speed effected the rate of release. At higher rotation speed, hydrodynamic effects were constant and variations of the rotation speed had no influence on the release rate of alendronic acid. Differences in the release rate were then determined by the properties of the tablets and equal results were obtained in 100 and 500 mL solvent volume. Therefore, dissolution testing of the samples was carried out in 100 mL solvent volume setting the rotation speed to 150 rpm.

The effect of temperature on the release of alendronic acid was investigated in the range from 31 to 39°C in 100 mL solvent volume at 150 rpm. The rate of release increased linearly with temperature but attenuated with time. Comparing the relative difference of the release of alendronic acid at 31 and 39°C, the highest increase was found after 5 s (2.5-fold) and declined to a 1.7- and 1.4-fold increase at 10 and 15 s, respectively. After 20 s the effect of temperature was not significant. Therefore, thermostatting of the solvent is necessary for the release tests. It also points out that temperature of the liquid used for oral intake of the tablet effects the amount of alendronic acid released into solution during deglutition.

Dissolution Testing

The latest commercially available lots of three European generic products and the original product were used for dissolution tests. Each blister pack contained four 70 mg alendronate tablets from which two tablets were taken for the tests. From each brand a total of six tablets was used for the release tests.

The tablets contain a mixture of active pharmaceutical ingredients, fillers, and disintegrants. Coming into contact with water, disintegrants cause swelling of the tablets leading to the release of the pharmaceutical ingredients. This process takes place immediately at contact with water and the release of water-soluble components is indicated by the formation of striae in solution. Then, after a short time, undissolved fines begin to separate from the tablet and were flushed away. The rate of dissolution depends on many variables such as chemical composition of the tablet, homogeneity, and physical properties, a direct result of the manufacturing process.

For deglutition of solid particles a mean duration of 5–10 s is assumed,[17] but scintigraphic measurements of the esophageal transit time performed on tablets of different shape and surface characteristics revealed differences up to 8 s.[18,19] For most subjects deglutition times up to 20 s were normal. Longer transit times were designated as esophageal stasis and are the exception. Therefore, in the current study, samples were collected after start for 20 s in intervals of 5 s.

The amount of alendronic acid released into solution increased approximately linearly with time, but unsteady with varying deviations in both directions, which reflects the inhomogenity of the individual pills and was different for each tablet. However, data averaging shows that the release of alendronic acid really follows a straight linear relationship (Fig. 3A). In the early state of dissolution the release rate of alendronic acid of the individual products is different and highest for the original product Fosamax (brand D). After 10 s about 7 mg alendronic acid or 10% of the nominal amount in the tablet has been released into solution. Concerning the complete dissolution profile, similar results were obtained (Fig. 3B). The formulations A, C, and D released 90% of the nominal amount into solution after about 2 min, whereas the difference to product B is now more pronounced and required 3.5 min to reach a release of 90%.

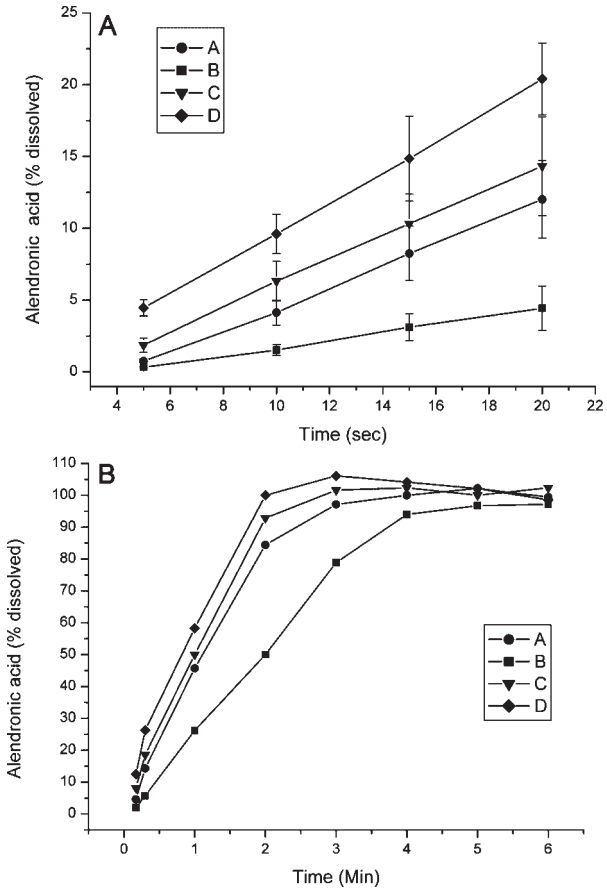

Figure 3. Mean dissolution profiles of the tested alendronate tablets showing (A) the early stage of dissolution and (B) complete range of dissolution.

Statistical Analysis

Because the dissolution profiles of the different brands in the early stage of dissolution are characterized by overlapping regions of variance (Fig. 3A), it is necessary to evaluate the significance of the measured differences by statistical methods.

This can be done either by parametric or nonparametric statistics. Parametric statistics requires normally distributed data. For the following tests each data set (tablet) was divided into data subsets (sampling time). The shape of the distribution of data is described by the kurtosis, a measure for the degree of peakedness of a distribution. In most cases a broad distribution function (platikurtic distribution) is received, which indicates frequently and modestly sized deviations.

Preliminary tests concerning the broadness and skewness of the distribution of data showed

normally distributed data sets. The actual distribution of the data subsets was tested by the test of Shapiro–Wilks for data sets with a limited number of data points. All data sets passed the test for normal distribution at a significance level of $p < 0.05$. Therefore, parametrical statistics can be applied for the statistical interpretation of the results of the dissolution experiments.

In the following, the mean values of the analytical results were compared by an independent *t*-test. Calculations were performed by the statistical software package of SPSS.

The dissolution profiles lying line by line were compared. The difference of the brands B to A and D to C was significant ($p < 0.05$), the difference of the brands A to C was only significant at the beginning of dissolution. Hence, the measured difference of all generic products to the original product is significant in the early state of dissolution and all generic products release definitely lower amounts of alendronic acid.

Over the complete range of dissolution, more than 85% of the label claim is released within 15 min, which indicates equal products according to the EMEA and FDA statements[20] for highly soluble pharmaceutical compounds. In addition to this simplified approach, a more precise analysis of the dissolution profiles was performed.

To describe the difference of dissolution profiles, Moore and Flanner[21,22] proposed the following equation (Eq. 1) to compare dissolution profiles:

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{1} w_t(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

The factor f_2 is calculated by the logarithmic transformation of the sum of the squared error and is a measure for the closeness between two profiles. R_t and T_t are the values at the compared time point t. n is the number of samples and w_t is an optional weight factor, which is set to 1 for current calculations.

Table 3. Similarity of Dissolution Profiles

	Similarity Factor f_2	
Compared Profiles	0–20 s	Complete Dissolution
D–A	66.8	58.5
D–B	55.7	37.6
D–C	74.7	70.5

The factor is 100 when the compared profiles are identical and approaches 0 as the dissimilarity increases. A f_2 value of 50 or greater (50–100) ensures sameness or equivalence of the two curves and, thus, the performance of the two products.

The generic products were compared to the original product and the results of the calculations are shown in Table 3. In the time interval from 0 to 20 s similarity f_2 of all formulations is above 50 and, therefore, they are equivalent to the original product. For the complete range of dissolution, in which the profiles of the formulations A and C stay close to the original product D (Fig. 3B), sameness of these products is confirmed. In contrast, product B diverges from the other profiles and similarity factor f_2 decreases to 37.6.

It should be mentioned that the results do not necessarily indicate lack of similarity,[23] but point out that a certain amount of difference exists for product B.

CONCLUSION

The release of alendronic acid from alendronate tablets of different brands was determined by *in vitro* dissolution tests at defined and reproducible conditions. In the early stage of dissolution, which covers the time interval for oral intake, the generic brands released significantly lower amounts of alendronic acid into solution than the original product. As a consequence, the potential health risk of all tested generic products during oral intake is lower than the original product. A clinical study for verification of these findings was not performed. The maximum amount that can be expected to be released into solution during deglutition is about 10% of the nominal amount in the tablet.

Over the complete range of dissolution, for one product the rate of dissolution and similarity f_2 is definitely lower, but according to the current EMEA and FDA statements, all products are equivalent.

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