

ORIGINAL RESEARCH

Comparative bone uptake study of alendronate sodium from vaginal suppositories prepared with polyethylene glycol and massa estarinum bases

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ABSTRACT: The aim of this study was to compare the bone uptake of alendronate sodium (ALD) from vaginal suppositories prepared with massa estarinum AB (ME) and polyethylene glycol 1500 (PEG) bases. For this purpose, ALD was radiolabeled with ^{99m}Tecnetium Pertechnetate (^{99m}Tc) by direct method. Radiochemical purity and stability of ^{99m}Tc-ALD was performed with chromatographic studies. ^{99m}Tc-ALD containing suppositories were prepared with ME and PEG bases. Physical properties of suppositories were evaluated. The physicochemical diffusion study was carried out to compare the release of ALD from different suppository bases. The bone uptake of ^{99m}Tc-ALD was observed by gamma scintigraphy studies. ^{99m}Tc-ALD containing suppositories were administrated to rabbits via vaginal route. The scintigraphic images were obtained with a gamma camera at different time intervals up to 240 minutes. According to our studies, radiochemical purity of ^{99m}Tc-ALD was observed more than 95% up to 6 hours. At 240 minutes of physicochemical diffusion studies, released ALD has 0.620 ± 0.091 mm and 10.465 ± 0.651 mm diameter zone from ME and PEG base suppositories respectively. According to the gamma scintigraphy studies, although no bone uptake observed after ME suppositories application, rabbit's bones were clearly visible after PEG suppositories applied. The results of physicochemical diffusion and gamma scintigraphy studies were found compatible in each other.

KEY WORDS: Alendronate sodium, bone uptake, vaginal suppository, massa estarinum, polyethylene glycol

INTRODUCTION

Bisphosphonates are used in the management of various disorders affecting the skeleton, including osteoporosis, metastatic bone disease and Paget's disease of bone. Bisphosphonates are selectively uptake at active bone sites, suppressed the osteoblast and osteoclast mediated bone resorption (1-4). Alendronate sodium (ALD) is a second generation amino bisphosphonates that selectively inhibits osteoclast-mediated bone resorption, increases bone mineral density and reduces the incidence of vertebral, hip and other fractures (5-7). Like all bisphosphonates, ALD is poorly absorbed from the gastrointestinal tract, with an oral bioavailability of around 0.9-1.8% (8). Its absorption is markedly reduced by food (9).

In recent years, there is a challenge for novel drug delivery systems to achieve improved bioavailability and safety. Various researchers have described new formulations to treat bone disease with minimal side effects and high efficiency. Because of the poor gastrointestinal absorption several administration routes have been attempted to enhance the bioavailability of bisphosphonates like intravenous, subcutaneous and intramuscular injections (10-13). Also Asikoglu et al. have demonstrated rectal absorption of ALD (14).

The unique properties like rich blood supply and the large surface area makes vagina an important area for systemic drug delivery (15). The advan-

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tages of vaginally applied dosage forms are avoidance of hepatic first-pass metabolism, decreasing of gastrointestinal and hepatic side effects; possible self-insertion and removal of the dosage form (15-17).

Suppositories generally consist of an active compound loaded into an inert matrix, which may be either a rigid or semi-rigid base. After administration, the role of the suppository is to liberate the active compound, either by melting due to body temperature or by dissolving in the local mucosal fluids, and then to release the active compound to produce a local effect or to move to the mucosal barriers into the systemic circulatory to produce a pharmacological effect (18).

Polyethylene glycol 1500 (PEG) is a water soluble suppository base which is widely used as excipients in a variety of pharmaceutical formulations, such as oral tablets and caplets, solutions and syrups, topical, rectal and vaginal preparations, ophthalmic, dental and parenteral systems (19, 20).

Massa estarinum AB (ME) is a synthetic suppository base that consists of a mixture of tri,di- and mono-glycerides of saturated fatty acids. ME has low melting point (29-31°C). ME is suitable for rapid absorption (19-21).

Vaginal uptake of pharmaceutical dosage forms can be investigated with gamma scintigraphy studies. During the last decades, very important improvements have been achieved in drug development using radiopharmaceuticals as tracers. Suitable radionuclide for scintigraphic studies can be chosen by considering factors such as the radiation energy, half-life, extent of particulate radiation, cost and availability. 99m Tc-pertechnetate (99m Tc) is the most popular radionuclide due to its versatile chemistry, near-ideal energy (140 keV), low radiation dose and short half-life (6 h) (22,23).

The aim of this study is to compare the bone uptake of ALD from ME and PEG suppositories in rabbits. The bone uptake of ALD was observed by gamma scintigraphy studies. For this reason ALD was radiolabeled with 99m Tc by direct method. Radiochemical purity and stability of 99m Tc-ALD was performed with radiochromatographic studies. 99m Tc-ALD containing suppositories were prepared with ME and PEG bases. Physical properties of suppositories were evaluated. The physicochemical diffusion study was carried out to compare the permeability of ALD from ME and PEG suppository bases. The 99m Tc-ALD containing ME and PEG suppositories were administrated to rabbits via vaginal route. The scintigraphic images were obtained with a gamma camera at different time intervals up to five hours.

MATERIALS AND METHODS

Materials

ALD was obtained as a gift from Arylsa Company. 99m Tc-sodium pertechnetate was obtained from Department of Nuclear Medicine of Ege University. Stannous chloride (Sigma) was used as reducing agent and ascorbic acid (Roche) was used as an antioxidant in labeling studies. The solutions were freshly prepared for each experiment under a nitrogen atmosphere. PEG 1500 (Henkel KGaA Düsseldorf, Germany) and ME-AB (Henkel KGaA Düsseldorf, Germany) were used as suppository bases. ALD was pharmaceutical grade and other chemicals used were analytical grade. The Animal Ethics Committee of the Ege University gave approval for the animal experi-

ments (Number: B.30.2.EGE.0.01.00.01/04-17/8, 2006). All experiments replicated at least six times. Results are reported as mean \pm standard error.

Radiolabeling Studies

ALD was directly labeled by 99m Tc with small modification on previously described (14). ALD (5 mg) was dissolved in saline (0.5 mL). 400 µg stannous chloride and 1 mg ascorbic acid were added to solution. Radiolabeling was performed with freshly eluted 37 megabecquerel (MBq) 99m Tc. The vials were shaken with vortex (Velp Scientifica) at 500 rpm for 30 seconds, filtered through a 0.22 µm pore size filter and incubated for 15 min at room temperature. The radiochemical purity was analyzed by chromatographic studies.

Lyophilized kits were prepared by mixing 5 mg ALD, 400 µg stannous chloride and 1mg ascorbic acid.

Radiochemical Purity and Stability Studies

Whatman No:3 chromatographic papers were used as stationary phases. Free 99m Tc was determined by using acetone as the mobile phase. Reduced/Hydrolyzed (R/H) 99m Tc was determined by using saline as mobile phase. % Radiochemical purity (RP) of 99m Tc-ALD was calculated from the following equation (Equation 1) by subtracting from 100 the sum of measured impurities percentages.

Equation 1:

$$RP \% = 100 - (Free \ ^{99m}Tc \% + R/H \ ^{99m}Tc \%)$$

After labeling ALD with 99m Tc, the preparation was left at room temperature for six hours. The labeling stability of the complex was evaluated by radio thin layer chromatography (RTLC) studies for every hour.

Preparation of Suppositories

Suppositories were prepared by using lyophilized kits which were prescribed above. Kits were labeled with 99m Tc in saline. The weight deviation of vaginal suppositories was determined previously with inactive experiments and amount of base was calculated. 99m Tc-ALD was mixed in melted PEG or ME and dispersed homogeneously. The resulting mixture was then poured into cylindrical plastic molds and allowed to cool at room temperature. Prepared suppositories were stored at 4°C until use. The final value of contents for each suppository was adjusted as 2.5 mg ALD and 1 mCi 99m Tc.

Physicochemical Properties of Suppositories

The suppositories were analyzed for their weight variation, hardness and melting time. Also 99m Tc-ALD loaded suppositories radioactivity was determined in a dose calibrator (Biodex Atomlab 100) to evaluate the drug content homogeneity of suppositories.

Physicochemical Diffusion Test of Suppositories

The physicochemical diffusion study was carried out to compare the permeability of ALD from different suppository bases (14). For this purpose, 0.5 g agar was dissolved in 24 mL water at 100°C water bath. After that the mixture was slightly cooled to lower degrees and the indicator (chloramphenicol solution) was added. The holes were opened on the agar with the lower part of hard gelatin capsule (size 4). 99m Tc-ALD loaded PEG and ME suppositories were placed in the holes and agar plates were incubated in the oven at 37 °C. The diffusion distances were determined by measuring the diameter of the colored

TABLE 1. Physicochemical properties of PEG and ME suppositories

	Weight Variations (g)	Hardness (a)	Melting Time (min)
PEG Suppository	0.112 ± 0.001	800±0.000	2.362± 0.005
ME suppository	0.098±0.002	600±0.000	1.573±0.003

zones at different time intervals (0.5, 1.0, 2.0, 2.5 and 3.0 h). Diffusion distance was calculated by the following equation (Equation 2).

Equation 2:

$$X = (C-D)/2$$

(X= diffusion distance, C= Zone diameter, D= Disc diameter)

In Vivo Studies

Female New Zealand White rabbits (2.5-3.0 kg) were used for animal studies. Experiments with rabbits were performed according to a protocol approved by Animal Ethics Committee of the Ege University.

During the scintigraphy studies rabbits were under anesthetized with Ketamine/Xylazin cocktail. 99m Tc-ALD (1mg/kg) loaded PEG and ME suppositories were inserted to the vagina of rabbits under anesthesia. After dosing, the vagina was glued together to prevent a leak of suppository. The scintigraphic images were obtained with a gamma camera (Apex SP-4, Elscint Ltd) equipped with a low-energy high-resolution collimator viewing the whole body of each rabbit in supine position. Serial static images were acquired in a 256×256 matrix for 300 sec. each, at 0, 60, 120, 180, 240 min after administration of radiolabeled formulations.

Statistical analysis

The calculation of means and standard deviations were made on Microsoft Excel. One-way Anova was used to determine statistical significance. Differences at the 95% confidence level ($p<0.05$) were considered significant.

RESULTS AND DISCUSSION

Radiolabeling Studies

The radiochemical purity of 99m Tc-ALD was found over 99% at room temperature immediately after incubation time. The complex was found stable at room temperature up to 6 h without any significant decrease in radiochemical purity ($p<0.05$). High radiochemical purity and stability makes 99m Tc-ALD suitable for investigating bone uptake with gamma scintigraphy.

Physicochemical Properties of Suppositories

Table 1 shows that all the suppository formulations satisfied the BP requirement. Due to 99m Tc-ALD loaded PEG and ME suppositories were prepared to use for in vivo studies with rabbit, prepared suppositories weight was around 100 mg. The average weight of suppositories prepared using PEG and ME were 0.112 and 0.098 kg respectively. Weight variations recorded were less than 5%. The suppositories prepared by PEG were found to be harder than ME suppositories as compared breaking forces. The hardness of ME suppositories was 600 g and the suppositories prepared with PEG were harder (800 g). Melting time for plain suppositories prepared was averagely all less than 30 minutes. Weight variation, hardness and melting time of PEG and ME suppositories are shown in Table 1.

99m Tc-ALD loaded suppositories radioactivity was determined in a dose calibrator and study indicated the homogeneous drug content

Physicochemical Diffusion Test of Suppositories

In order to investigate the release of ALD from different suppository bases, two different suppository formulations were prepared by using lipophilic ME and hydrophilic PEG. Diffusion distance of ALD was found significantly different for PEG and ME suppositories ($p<0.05$). Diffusion zone of ALD from ME suppositories was 0.620 ± 0.091 mm while 10.465 ± 0.651 mm diameter zone was observed with PEG suppositories (Table 2).

TABLE 2. The photograph of colored zones and average diffusion distances of 99m Tc-ALD from PEG and ME suppositories in chloramphenicol containing agar at 30 and 240 min.

Suppository	30 min		240 min
	Photographs of colored zones	Diffusion Distance Average (mm)	
PEG Suppository		4.015 ± 0.284	
ME Suppository		0.510 ± 0.124	

A physicochemical characteristic of a drug is important in selecting the suppository base and in anticipating drug release from that base. Lipophilic drug, in a fatty suppository base has fewer tendencies to escape to the surrounding aqueous fluids. Thus water-soluble salt are preferred in fatty base suppository but from PEG suppositories, drug release depends on the base dissolving rather than melting so this base can be used for both water-soluble and oil-soluble drugs (18).

PEG is a water soluble polymer that often melts at temperatures higher than the physiological temperature of 37°C . Consequently, when used in vaginal or rectal formulations, the drug is released gradually, as a result of the progressive dissolution of the PEG excipients in an aqueous dissolution medium. Melting of a fatty base is followed by several successive mechanistic steps, such as spreading of the molten mass, particle dispersion, passage across the interface and finally dissolution of the drug in the hydrophilic fluids (18).

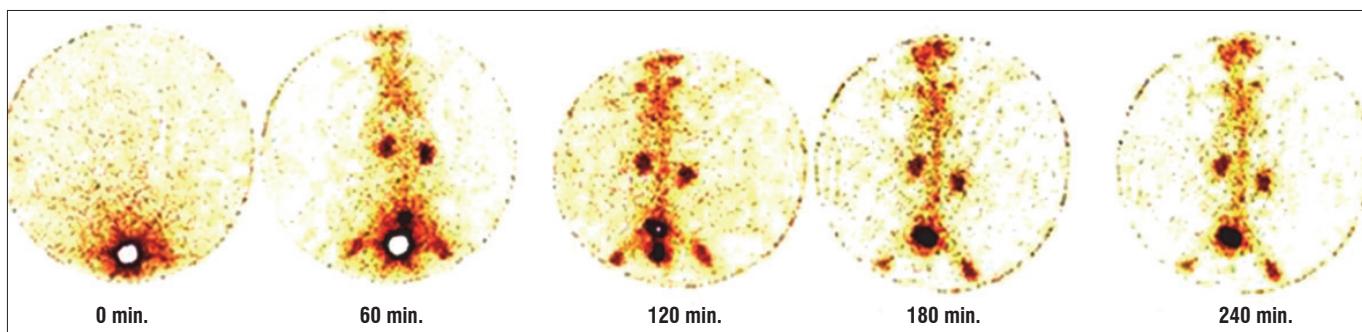


FIGURE 1. Scintigraphic images of ^{99m}Tc -ALD suppositories prepared with PEG at 0, 60, 120, 180 and 240 min. after administration.

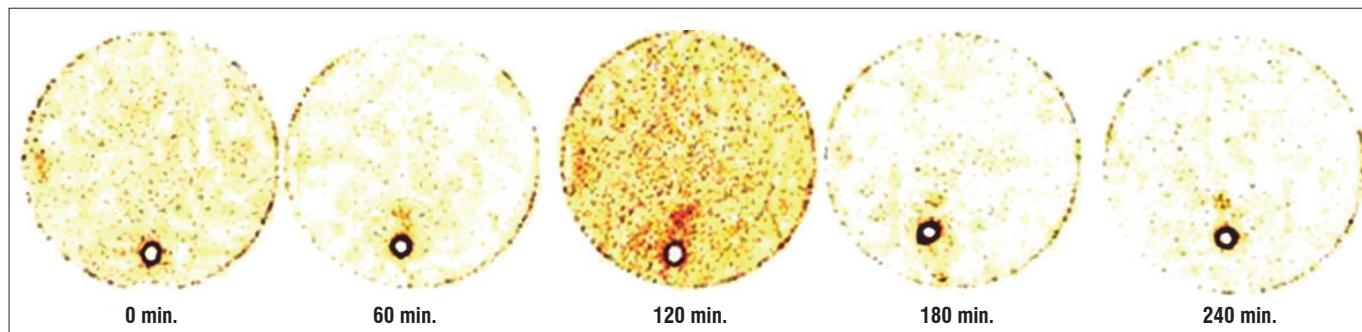


FIGURE 2. Scintigraphic images of ^{99m}Tc -ALD suppositories prepared with MEG at 0, 60, 120, 180 and 240 min. after administration.

According to the physicochemical diffusion studies bases play an important role for drug release from suppositories.

In Vivo Studies

Selection of a suitable base cannot be made in the absence of knowledge of the physicochemical properties and intrinsic thermodynamic activity of the drug substance which is to be incorporated into the suppository. Other drug-related factors can affect the base selection.

The bone uptake of ^{99m}Tc -ALD following intravaginal administration of PEG and ME suppositories was investigated on static images (Figure 1-2). Scintigraphic images clearly demonstrated the bone uptake of ^{99m}Tc -ALD from PEG suppository after intravaginal administration. ^{99m}Tc -ALD was not released from ME suppository, and the radioactivity stayed in the application point for 240 minute. The differences in the percentage of radioactivity of ME and PEG suppositories remaining in vagina were found significantly different ($P < 0.05$).

Bioavailability depends not only the formulation. The factors which may affect drug release and dissolution, are potential barriers to the passage of dissolved drug into venous blood or lymph also exist in the unstirred water layer, the mucous lay-

er, the cellular structure of the mucous membrane and the walls of lymph vessels and blood capillaries.

CONCLUSION

In this study, ^{99m}Tc -ALD loaded PEG and ME suppositories were prepared and bone uptake of ALD via vaginal route was estimated by gamma scintigraphy studies. Gamma scintigraphy studies demonstrated the impotence of radiolabeled drugs to investigate the systemic absorption via vaginal application. According to the results of our study, suppository base play major role in drug release and should be selected carefully. With vaginal suppositories of ALD, the undesired side effects, poor gastrointestinal absorption and food interaction can be eliminated. Therefore PEG suppositories of ALD are promising for bone targeting for women.

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Polietilen glikol ve massa estarinum sivağları kullanılarak hazırlanan vajinal supozituvardan salınan alendronat sodyumun kemik tutulumunun karşılaştırmalı incelenmesi

ÖZET: Çalışmamızın amacı, massa estarinum AB (ME) ve polietilen glikol 1500 (PEG) sivağları kullanılarak hazırlanan vajinal supozituvardan alendronat sodyumun (ALD) kemik tutulumunun karşılaştırmalı olarak incelenmesidir. Bu amaçla, ALD 99m Teknesyum Perteknetat (99m Tc) ile direk metot kullanılarak işaretlenmiş, 99m Tc-ALD'in radyokimyasal saflık ve stabilitesi kromatografik çalışmalar ile incelenmiştir. 99m Tc-ALD içeren supozituvalar ME ve PEG sivağları kullanılarak hazırlanmış, supozituvaların fiziksel özelliklerini incelenmiştir. ALD'in farklı supozituvar sivağlarından salımını karşılaştırmalı olarak incelemek amacıyla fizikokimyasal difüzyon çalışmaları yapılmıştır. 99m Tc-ALD'in kemik tutulumu yapılan gama sintigrafi çalışmaları ile belirlenmiştir. 99m Tc-ALD içeren ME ve PEG sivağları kullanılarak hazırlanan supozituvalar tavşanlara vajinal yoldan uygulandıktan sonra gama kamera ile 240 dakika boyunca farklı zaman aralıklarında sintigrafik görüntüler alınmıştır. Yapılan çalışmalarla göre, 99m Tc-ALD'in radyokimyasal safliğinin 6 saat boyunca %95'in üzerinde olduğu belirlenmiştir. Fizikokimyasal difüzyon çalışmalarında 240 dakika sonunda; ME sivası ile hazırlanan supozituvardan salınan ALD $0,620 \pm 0,091$ mm çapta zon oluştururken, PEG ile hazırlanan supozituvardan salınan ALD $10,465 \pm 0,651$ mm çapta zon oluşturmuştur. Yapılan gama sintigrafi çalışmalarına göre, ME sivası kullanılarak hazırlanan supozituvalar uygulanan tavşanlarda herhangi bir kemik tutulumu gözlenmemekten, PEG sivası kullanılarak hazırlanan supozituvaların uygulandığı tavşanlarda belirgin kemik tutulumu gözlenmiştir. Sonuç olarak fizikokimyasal difüzyon ve gama sintigrafi çalışmalarının sonuçlarının birbirleri ile uyumlu olduğu tespit edilmiştir.

ANAHTAR KELİMELER: Alendronat sodyum, kemik tutulumu, vajinal supozituvar, massa estarinum, polietilen glikol

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