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-

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 $^{99m}{	ext{Tc}}$ as $^{99m}{	ext{Tc}}$-pertechnetate ($^{99m}{	ext{Tc}}$) by direct method. Radiochemical purity and stability of $^{99m}{	ext{Tc}}$-ALD was performed with chromatographic studies. $^{99m}{	ext{Tc}}$-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 $^{99m}{	ext{Tc}}$-ALD was observed by gamma scintigraphy studies. $^{99m}{	ext{Tc}}$-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 $^{99m}{	ext{Tc}}$-ALD was observed more than 95% up to 6 hours. At 240 minutes of physicochemical diffusion studies, released ALD has $0.620 \pm 0.091$ mm and $10.465 \pm 0.651$ 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

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tages of vaginally applied dosage forms are avoidance of he-
aptic 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 pharma-
ceutical formulations, such as oral tablets and caplets, solu-
tions and syrups, topical, rectal and vaginal preparations, oph-
thalmic, 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 saturat-
ed fatty acids. ME has low melting point (29-31°C). ME is suit-
able for rapid absorption (19-21).

Vaginal uptake of pharmaceutical dosage forms can be inves-
tigated with gamma scintigraphy studies. During the last dec-
ades, very important improvements have been achieved in
drug development using radiopharmaceuticals as tracers.
Suitable radionuclides for scintigraphic studies can be chosen
by considering factors such as the radiation energy, half-life,
extent of particulate radiation, cost and availability. 99mTc-
netium pertechnetate (99mTc) 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 99mTc by direct method.
Radiochemical purity and stability of 99mTc-ALD was per-
formed with radiochromatographic studies. 99mTc-ALD con-
taining 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
bas.
The 99mTc-ALD containing ME and PEG suppositories were
administered 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. 99mTc-sodi-
um 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 supposi-
tory bases. ALD was pharmaceutical grade and other chemi-
cals used were analytical grade. The Animal Ethics Committe
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 ex-
periments replicated at least six times. Results are reported as
mean ± standard error.

Radiolabeling Studies

ALD was directly labeled by 99mTc 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) 99mTc. The vials were shaken
with vortex (Velp Scientifica) at 500 rpm for 30 seconds, fil-
tered through a 0.22μm pore size filter and incubated for 15
min at room temperature. The radiochemical purity was ana-
yzed by chromatographic studies.

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

Radiochemical Purity and Stability Studies

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

$$\text{Equation 1: } \text{RP} \% = 100 - (\text{Free } 99mTc \% + \text{R/H } 99mTc \%)$$

After labeling ALD with 99mTc, 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 99mTc in saline.
The weight deviation of vaginal suppositories was determined
previously with inactive experiments and amount of base was
calculated. 99mTc-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 99mTc.

Physicochemical Properties of Suppositories

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

Physicochemical Diffusion Test of Suppositories

The physicochemical diffusion study was carried out to com-
pare 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). 99mTc-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
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:**

\[ X = \frac{(C-D)}{2} \]

\(X=\) 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 anesthetize with Ketamine/Xylazin cocktail. 99mTc-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, Elsclint 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 99mTc-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 99mTc-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 99mTc-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 was averagely all less than 30 minutes. Weight variation, hardness and melting time of PEG and ME suppositories are shown in Table 1.

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 queues 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).

**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 1.** Physicochemical properties of PEG and ME suppositories

<table>
<thead>
<tr>
<th></th>
<th>Weight Variations (g)</th>
<th>Hardness (a)</th>
<th>Melting Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG Suppository</td>
<td>0.112 ± 0.001</td>
<td>800±0.000</td>
<td>2.362± 0.005</td>
</tr>
<tr>
<td>ME Suppository</td>
<td>0.098±0.002</td>
<td>600±0.000</td>
<td>1.573±0.003</td>
</tr>
</tbody>
</table>

**TABLE 2.** The photograph of colored zones and average diffusion distances of 99mTc-ALD from PEG and ME suppositories in chloramphenicol containing agar at 30 and 240 min.
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 layer, 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 SVAĞLARI KULLANILARAK HAZIRLANAN VAJINAL SUPOTUZVARLARDAN SALINAN ALENDRONAT SODYUMUN KEMİK TUTULMUNUN KARŞILAŞTIRMALI İNCelenMESI

ÖZET: Çalışmamızın amacı, massa estarinum AB (ME) ve polietilen glikol 1500 (PEG) sıvı yapıştılar vajinal suppozitvarlardan alendronat sodyumun (ALD) kemik tutulumunun karşılaştırılması incelenmesidir. Bu amaçla, ALD $^{99m}$Teksnymyum Perteknetat ($^{99m}$Tc) ile direk metot kullanarak radyokimyasal saflık ve stabilitesi kromatografik çalışmaları ile incelenmiştir. $^{99m}$Tc-ALD içeren supozitvarlar ME ve PEG sıvı yapıştılar hazırlanmış, supozitvarların fiziksel özellikleri incelenmiştir. ALD’ın farklı supozitvar sıvıitasından salınımı karşılaştırılmış olarak incelenmek amacı ile fizikokimyasal difüzyon çalışmaları yapılmıştır. $^{99m}$Tc-ALD’ın kemik tutulumu yapılan gama sintigrafi çalışmaları ile belirlenmiştir. $^{99m}$Tc-ALD içeren ME ve PEG sıvı yapıştılar hazırlanan supozitvarlar tavanlar vajinal yoldan uygulandıktan sonra gama kamera ile 240 dakika boyunca farklı zaman aralıklarında sintigrafik görüntülar alınmıştır. Yapılan çalışmalarla göre, $^{99m}$Tc-ALD’ın radyokimyasal saflığının 6 saat boyunca %95’in üzerinde olduğu belirlenmiştir. Fizikokimyasal difüzyon çalışmaları 240 dakika sonunda; ME sıvı ile hazırlanan supozitvarlardan salinan ALD 0,620±0,091 mm çapta zon oluşturken, PEG sıvıda hazırlanan supozitvarlardan salinan ALD 10,465±0,651 mm çapta zon oluşmuştur. Yapılan gama sintigrafi çalışmaları göre, ME sıvıı kullanılanlar hazırlanan supozitvarların tavanlarında belirgin kemik tutulumu gözlenmiştir. Sonuç olarak fizikokimyasal difüzyon ve gama sintigrafi çalışmalarının sonuçlarının birbirleri ile uyumu olduğu tespit edilmiştir.

ANAHTAR KELİMELER: ALENDRONAT SODYUM, KEMİK TUTULUMU, VAJINAL SUPOTUZVAR, MASSA ESTARINUM, POLIETILEN GLIKOL

REFERENCES