Evaluation bone uptake of alendronate sodium via vaginal route by gamma scintigraphy, Vaginal uptake of alendronate sodium

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Alendronate sodium (ALD) is a second generation amino bisphosphonate that use for treatment of bone diseases. Since ALD is poorly absorbed from the gastrointestinal tract and its absorption is markedly reduced by food, the aim of this study is to evaluate the bone uptake of ALD through vaginal route. To evaluate the bone uptake of ALD by gamma scintigraphy, ALD was radiolabeled with Technetium-99m (^{99m}Tc). Vaginal suppositories and injectable solution of ^{99m}Tc-ALD was prepared and gamma scintigraphy studies were performed with intravaginally and intravenously ^{99m}Tc-ALD applied rabbits. The results were revealed that ALD was successfully labeled with ^{99m}Tc. After intravaginal and intravenous application, ^{99m}Tc-ALD showed a high uptake in bone. Despite accumulation times and uptake ratios showed differences between administration routes, our preliminary observations suggest that the intravaginal route appears to be a viable alternative for ALD application and should be evaluated in future clinical studies.

Key words: Alendronate sodium – Bone uptake – Gamma scintigraphy – Intravaginal application – Technetium-99m.

Bisphosphonates (BPs) are stable analogs of pyrophosphates (P-O-P) and therapeutic agents for the treatment of bone diseases. They are strongly attracted to the bone where they influence the calcium metabolism, mainly by inhibition of the osteoclast-mediated bone resorption [1, 2]. Bisphosphonates have been extensively applied in medicine, particularly in the fields of osteology, orthopedics and surgery. Over the past 12 years bisphosphonates have played a major role in the treatment of postmenopausal osteoporosis [1, 3]. The basic structure of bisphosphonates allows many possible variations, by changing the two lateral chains (R1-R2) on the carbon atom [4]. The various bisphosphonates are distinguished one from another by the ligands R1 and R2. Bisphosphonates are including ALD, etidronate, pamidronate, ibandronate, risedronate, clodronate, tiludronate, and zoledronate, which are variously used as the free acid or as the sodium salt [1].

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]. The oral bioavailability of 10 mg ALD tablet, taken with plain water after an overnight fast and 2 h before ingestion of the first food of the day, is 0.8 % in woman. When the drug is taken 30 or 60 min before breakfast, its oral bioavailability is reduced to approximately 0.5 %. The drug's bioavailability is reduced to approximately 0.3 % when ALD is taken with coffee or orange juice instead of plain water and negligible when taken up to 2 h after a standardized breakfast [10]. ALD is generally well tolerated. The most common adverse effect is upper gastrointestinal irritation, resulting in nausea, vomiting, abdominal pain, dyspepsia, esophagitis, and esophageal reflux. Esophageal ulcers, stricture and erosion have also been reported [7, 11, 12]. It appears that a once weekly dosage with a large dose (70 mg) produces fewer gastrointestinal adverse effects than daily administration of a low dose [13]. To minimize adverse gastrointestinal effects, the tablets should be swallowed with a large glass of water; the patient should remain standing or seated upright and should not lie down for at least 30 min after eating [14, 15].

In recent years, there is a challenge for novel drug delivery systems to achieve improved bioavailability and safety. Various methods have been used to improve the bioavailability of bisphosphonates are described. Because of the poor gastrointestinal (GI) absorption several administration routes have been attempted to enhance the bioavailability of bisphosphonates like intravenous, subcutaneous and intramuscular injections [16-19].

The vagina is an important area of the reproductive tract and serves as a potential route for drug administration. The anatomical position, the rich blood supply and the large surface area of the vagina predestines it as an application site for systemic drug delivery. Despite the fact that vaginal delivery is only available for females there are numbers of advantages like: the avoidance of hepatic first-pass metabolism, decreasing of gastrointestinal and hepatic side effects. It overcomes the inconvenience caused by pain, tissue damage and probable infection by parenteral routes. Another advantage is the possible self-insertion and removal of the dosage form [20].

Gamma scintigraphy is one of the most popular methods to investigate the GI performance of pharmaceutical dosage forms. This non-invasive technique gives information about integrity, dispersion or release characteristics of the radiolabeled delivery system. Choice of a suitable radionuclide for scintigraphic studies can be ascertained by considering factors such as the radiation energy, half-life, extent of particulate radiation, cost and availability. Technetium-99m (^{99m}Tc) is the most popular radionuclide with its versatile chemistry, near-ideal energy (140 keV), low radiation dose and short half-life (6 h) [21, 22].

The purpose of the present study was to radiolabeled of ALD with ^{99m}Tc, evaluate radiochemical purity and compare bone uptake of ALD which applied via vaginal and intravenous route in rabbits. Vaginal application potential of ALD was evaluated.

I. MATERIALS AND METHODS

1. 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 labe-

ling studies. The solutions were freshly prepared for each experiment under a nitrogen atmosphere. PEG 1500 (Henkel KGaA Düsseldorf, Germany) was used as suppository base. ALD was pharmaceutical grade and other chemicals used were analytical grade. The Animal Ethics Committee of the Ege University gave approval for the animal experiments (Number: B.30.2.EGE.0.01.00.01/04-17/8, 2006). Results are reported as mean ± standard error.

2. Radiolabeling studies

ALD was directly labeled by ^{99m}Tc with small modification on previously described [23]. To investigate the optimum conditions', radiolabeling was tested with different concentrations of reducing agent, stability of the complex was evaluated in the absence and presence of antioxidant agent. Radiochemical purity was determined with radio thin layer chromatography (RTLC) analysis. Ready to use freeze dry kit was formulated with optimum labeling conditions.

Briefly, ALD (5 mg) was dissolved in saline (0.5 mL). To this solution 400 μg of stannous chloride which dissolved in water distillate (1 mg/1 mL) and 1mg ascorbic acid were added under an atmosphere of bubbling nitrogen. Radiolabeling was performed with ^{99m}Tc (37 MBq) in saline (0.1 mL) and solution was allowed to stand at room temperature for 15 min. After incubation period, the solution filtered from a cellulose acetate filter (0, 22 μm pore size) into a vial prior to radiochemical analysis. The labeling stability of the complex was evaluated by RTLC studies.

Radiochemical analysis of the complex was performed with RTLC studies as described elsewhere [23]. 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. Five microlitres of samples were stopped on the chromatographic papers, air dried, and developed in acetone and saline. The chromatography papers were dried, cut into 1 cm segments and counted by using a gamma counter. Radiochemical purity (RP) of ^{99m}Tc-ALD was calculated from the following equation by subtracting from 100 the sum of measured impurities percentages:

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

3. Preparation of ^{99m}Tc-ALD lyophilized kit

After observing the effect of different parameters on labeling, subsequently kits prepared by mixing 5 mg ALD, 400 μg stannous chloride and 1mg ascorbic acid. Lyophilized kits were stored at 4-7 °C until use. At the time of use the kits were reconstituted with 37 MBq of ^{99m}Tc-pertechnetate and the radiochemical purity was analyzed by RTLC.

4. Preparation of ^{99m}Tc-ALD vaginal suppositories

Suppositories were prepared by using lyophilized kits which prescribed above. 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 1500 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.

5. Preparation of ^{99m}Tc-ALD intravenous solution

^{99m}Tc-ALD intravenous solution was prepared by labeling lyophilized kits with ^{99m}Tc. The mixture dispensed in syringes equally to make each contains 2.5 mg ALD and 1 mCi ^{99m}Tc which are equal with ^{99m}Tc-ALD suppositories.

6. *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. Suppositories containing ^{99m}Tc-ALD (1 mg/kg) were inserted to the vagina of rabbits under anesthesia. After dosing, the vagina was glued together to prevent a leak of suppository. ^{99m}Tc-ALD solution (1 mg/kg) was administered in to the ear vein of the rabbit. 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 s each, at 0, 60, 120, 180, 240 min after administration of radiolabeled formulations.

7. Statistical analysis

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

II. RESULTS

1. Radiolabeling studies

Radiochemical purity of the ^{99m}Tc-ALD was assessed by RTLC studies. Two solvent systems were used to distinguish and quantify the amounts of radioactive contaminants (Free ^{99m}Tc, R/H ^{99m}Tc).

In RTLC using acetone as the solvent, free ^{99m}Tc moved with the solvent front, while ^{99m}Tc-ALD and R/H ^{99m}Tc remained at the spotting point. R/H ^{99m}Tc was determined by using saline as the mobile phase where the R/H ^{99m}Tc remained at the point of spotting while free ^{99m}Tc and ^{99m}Tc-ALD moved with the solvent front.

Five milligrams ALD was labeled with ^{99m}Tc using 400 μg stannous chloride (1 mg/1 mL) in water distillate as the reducer and 1 mg of ascorbic acid as the stabilizer. The radiochemical purity of ^{99m}Tc-ALD was found over 99 % at room temperature immediately after incubation time.

Different batches of the kits were used for chromatographic studies. There were no significantly differences in the radiochemical purity of kits. It was observed that in kits the unbound $^{99m}TcO_4^-$ was 0.01 % ± 0.004, the H/R ^{99m}Tc was 0.60 % ± 0.06. This was also substantiated in imaging studies. No tracer concentration was seen in the salivary glands, thyroid gland or stomach, suggesting the absence of significant unbound $^{99m}TcO_4^-$ in the preparation. ^{99m}Tc-ALD was stable up to 6 h without any significant decrease in the radiochemical purity.

2. *In vivo* studies

The bone uptake of ^{99m}Tc-ALD following intravenous and intravaginal administrations was assessed on static images. As expected, the bone uptake of ^{99m}Tc-ALD following intravenous administration was faster and more than intravaginal administration. For quantitative evaluation, regions of interest were drawn around the kidney, urinary bladder, liver, vertebra, epiphysis, femur, thorax, shoulder of the rabbits (*Figure 1*). Bone and other organs uptake of rabbits were calculated dividing by the ratio of decay corrected counts per pixel in the region of target to ratio of decay corrected counts per pixel in the region of soft tissue.

Scintigraphic images are clearly demonstrated the bone uptake of ^{99m}Tc-ALD from intravaginal and intravenous administration routes (*Figure 2*).

The bone uptake of ^{99m}Tc-ALD which applied via intravenous and intravaginal routes are shown in *Figures 3 and 4*.

Systemically available ALD is either taken up by bone tissue or excreted by the kidneys. The kidney and liver uptake of ^{99m}Tc-ALD after both administration routes are shown in *Figure 5*.