Osteotropic Drug Delivery System (ODDS) Based on Bisphosphonic Prodrug. V. 1) Biological Disposition and Targeting Characteristics of Osteotropic Estradiol

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An osteotropic drug delivery system (ODDS) based on a bisphosphonic prodrug has been developed for 17β-estradiol (E2) to improve patient compliance in estrogen replacement therapy of postmenopausal osteoporosis. The biological disposition and the targeting efficiency of a bisphosphonic prodrug of E2, disodium [17β-(3'-hydroxy-1',3',5'-estratrienyl)oxycarbonylpropyl carboxamidomethylene]bisphosphonate (E2-BP), was investigated in ovariectomized rats. After intravenous injection, E2-BP was rapidly taken up into the bone and subsequently cleared from the bone at a half-life of 13.5 d. The bone concentration of regenerated E2 was maintained throughout 28 d. In contrast, E2 injected intravenously showed extremely low bone distribution and rapid clearance from the bone, and E2 administered orally showed even lower bone distribution. Therapeutic availability (TA) and drug targeting index (DTI), which were calculated on the basis of the AUCs for E2 in the bone and plasma after injection of E2-BP and E2, were 64.6 and 451, respectively. These results suggest that ODDS has a potential to improve not only the apparent potency but also the therapeutic index of E2. As compared with the conventional estrogenic products, E2-BP should improve patient compliance with lower adverse effects and less frequent medication in long-term estrogen replacement therapy.

Key words estradiol; osteotropic drug delivery system; prodrug; bone; drug targeting index; therapeutic availability

Osteoporosis is one of the most formidable health problems in advanced society. For example, in the United States it is responsible for 1.5 million fractures each year at an annual cost of $10 billion.2) Since a reduction in estrogen production after menopause is thought to be associated with the accelerated bone loss in postmenopausal women,3) estrogen replacement therapy is an effective treatment for prevention of fractures in postmenopausal osteoporosis.5) It has become apparent that the anti-osteoporotic effect of estrogen is based on its direct action on the bone.6)7) Since estrogen receptors are present in many tissues, however, the estrogen distributed in tissue other than the bone may cause a number of unwanted side effects in estrogen therapy of postmenopausal osteoporosis. In fact, use of estrogen replacement therapy after menopause has been related to increased incidence of uterine and breast cancer, hypertension, thromboembolic disease, liver and gallbladder diseases, and edema.8)9)10)
Bone targeted drug delivery, therefore, has the potential to improve the therapeutic index of estrogen by improving the potency and decreasing the toxicity.

We recently proposed an osteotropic drug delivery system (ODDS) based on a bisphosphonic prodrug as a novel method for site-specific and controlled delivery of drugs to the bone.11)12) This unique approach is based on high affinity of the prodrug to a mineral component called hydroxyapatite in the bone through bisphosphonic promoiety.13) E2-BP (disodium [17β-(3-hydroxy-1',3',5'-estratrienyl)oxycarbonylpropyl carboxamidomethylene] bisphosphonate) (Fig. 1), a bisphosphonic prodrug of 17β-Estradiol (E2), has been developed as the first example of ODDS of an antosteoporotic drug. After systemic administration, E2-BP is rapidly taken up by the bone according to its physicochemical property, and is then subject to enzymatic and/or chemical hydrolysis to generate E2 in the bone. The same moiety is rapidly cleared from the periphery because of its increased hydrophilicity due to introduction of two phosphonate groups. As expected from the concept of ODDS, E2-BP showed excellent therapeutic efficacy and a long-acting pharmacological effect superior to E2 in ovariectomized rats.13)

In this study, the biological disposition of E2-BP after intravenous injection into rats was investigated to determine its targeting characteristics to the bone. Targeting efficiency was assessed quantitatively using the drug targeting index (DTI) and therapeutic availability (TA) proposed by Hunt et al.14) In addition, the disposition of E2-BP was compared with that of E2 orally administered, which exhibited a narrower therapeutic window than E2-BP.13)

MATERIALS AND METHODS

Materials 17β-Estradiol (E2) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Disodium [17β-(1',3',5'-estratrienyl)oxycarbonylpropyl carboxamidomethylene]bisphosphonate(E2-BP, Fig. 1) was synthesiz-

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Fig. 1. Chemical Structure of Disodium [17β-(3-hydroxy-1',3',5'-estratrienyl)oxycarbonylpropyl carboxamidomethylene]bisphosphonate (E2-BP)

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ed as previously reported. Briefly, 17β-oestradiol-3-benzoate was coupled with triethyl hemiglutarylarnidomethylene bisphosphonate to yield carboxylic ester bond. The phenol ester and phosphonate ester in this intermediate were selectively hydrolyzed by methanolic potassium hydrogen carbonate and trimethylsilyl iodide, respectively. E₂-BP was then prepared as disodium salt with sodium acetate. All other reagents and solvents were of analytical grade and purchased from commercial vendors.

Animals and Treatment  Female Wistar rats (10 weeks old) were purchased from Japan Clea (Tokyo, Japan). The animals were housed under standard conditions (temperature: 23 ± 2°C, humidity: 55 ± 5%) and maintained under a 12-h light/dark cycle with free access to water and food. To evaluate the bone and plasma concentrations of exogenous E₂, rats were bilaterally ovariectomized under pentobarbital anesthesia (40 mg/kg, i.p.). On day 14 after ovariectomy, E₂-BP was injected as 5% glucose solution into rats via the tail vein at doses of 1 mg/kg. E₂ was injected in the same manner at a dose of 0.48 mg/kg (equimolar to 1 mg/kg of E₂-BP). Additionally, E₂ suspended in 0.5% methyl cellulose was given orally at doses of 1 mg/kg. At various time periods, rats (five per group) were anesthetized with diethyl ether. The blood samples were withdrawn from the heart and then both femurs were excised. Each femur was weighed, cleaned off the soft tissues and frozen in liquid nitrogen to avoid postcollection degradation of E₂-BP. After lyophilization, the femurs were weighed and stored in a freezer until assay. An aliquot (0.1 ml) of the plasma was stored in a freezer until assay. Care and treatment of experimental animals were as approved by the Animal Care and Use Committee at Fujisawa Pharmaceutical Company, Ltd.

Bone and Plasma Analysis  In order to analyze E₂-BP and regenerated E₂ in the femur, the femur was cut into several pieces and homogenized in distilled water (4 µl/1 mg). Homogenized femur samples and plasma samples were assayed for E₂ and E₂-BP by radioimmunoassay (RIA) of E₂. Since the cross-reactivity of E₂ antibody for E₂-BP was 0.22% at a concentration above 5 ng/ml, E₂ in homogenized femur and plasma samples was assayed directly by RIA. For analysis in the plasma, E₂-BP in each plasma sample was hydrolyzed in 1 N NaOH at 60 °C for 1 h. After hydrolysis, the sample was neutralized with HCl and total E₂ (E₂ existing in the plasma + E₂ regenerated from E₂-BP in 1 N NaOH) was extracted into diethyl ether. An aliquot of the organic layer was dried under a nitrogen stream, dissolved in control serum and assayed in duplicate by RIA. For the analysis of E₂-BP in the femur, homogenized femur samples were dissolved in 6 N HCl at 60 °C for 1 h. During this procedure, E₂-BP was completely hydrolyzed to E₂ (data not shown). After neutralization with NaOH, total E₂ was extracted with isoamylalcohol and assayed in a similar manner to the plasma assay procedure. The amounts of E₂-BP existing in plasma and femur were calculated as the difference between total E₂ and E₂.

RIA  RIA commercial kits, a solid-phase 125I-RIA designed for quantitative measurement of E₂ in serum were purchased from Japan DPC Corporation (Tokyo). Each kit is equipped with human serum-based standards having E₂ concentrations ranging from 5 to 500 pg/ml. The cross-reactivity for estradiol and estrone to the E₂ antibody has been reported to be 0.235 and 1.3%, respectively (technical information from Japan DPC Corporation).

Data Analysis  The plasma concentration and bone concentration of E₂-BP and E₂ was normalized on the basis of administration dose. The areas under the plasma concentration-time curves (AUCs) up to the last measured time point were calculated using the trapezoidal method. Drug targeting index (DTI) is defined as the ratio of drug delivered to response and toxicity sites when a targeted delivery system is applied, divided by the same ratio when a non-targeted delivery system is used. Practically, as shown in Eq. 1, DTI was calculated as the AUC ratio of E₂ regenerated in the bone and plasma after intravenous administration of E₂-BP, divided by the same ratio after intravenous administration of E₂:

\[
DTI = \frac{\frac{AUC_{\text{regenerated E}_2, \text{ bone}}}{AUC_{\text{regenerated E}_2, \text{ plasma}}}}{\frac{AUC_{\text{E}_2, \text{ bone}}}{AUC_{\text{E}_2, \text{ plasma}}}}_{\text{E}_2-\text{BP administration}}
\]

Therapeutic availability (TA) is the ratio of the dose fraction reaching response sites when a targeted delivery system is applied to the amount reaching the same sites when a non-targeted delivery system is used. In this study, according to Eq. 2, TA was calculated as the ratio of AUC values of E₂ in bone when E₂-BP and E₂ was administered intravenously:

\[
TA = \frac{\frac{AUC_{\text{regenerated E}_2, \text{ Bone}}}{AUC_{\text{E}_2, \text{ Bone}}}_{\text{E}_2-\text{BP administration}}}{\frac{AUC_{\text{E}_2, \text{ Bone}}}{AUC_{\text{E}_2, \text{ Bone}}}}_{\text{E}_2 \text{ administration}}
\]

RESULTS

Bone Concentration of E₂-BP and E₂  Figure 2 depicts the bone concentration-time curves of E₂-BP and regenerated E₂ in rats after intravenous injection of E₂-BP together with those of E₂ after intravenous and oral administration of E₂. At 1 h after injection of E₂-BP, the highest bone concentration of E₂-BP was observed. There-

![Fig. 2. Bone Concentration–Time Curves after Administration of E₂-BP and E₂ to Rats](image-url)

Each point represents the mean value with S.E. of five rats. Key: ○, E₂-BP after intravenous injection of E₂-BP with 1 mg/kg; ▲, E₂ after intravenous injection of E₂-BP with 0.48 mg/kg; ■, E₂ after oral administration of E₂ with 1 mg/kg.
after, the bone concentration of E$_2$-BP decreased at a half-life of 13.5 d up to 28 d. The bone concentration of regenerated E$_2$ was maintained for a long time although its levels were lower than those of E$_2$-BP by a factor of more than 100. In contrast with regenerated E$_2$ after injection of E$_2$-BP, intravenously injected E$_2$ showed extremely low bone distribution and rapid clearance from the bone to below the detection limit (1 × 10$^{-5}$% of dose/g) at 24 h. The bone concentration of E$_2$ after oral administration was much lower than that after intravenous administration and had dropped below the detection limit at 48 h.

**Plasma Concentration of E$_2$-BP and E$_2$** Figure 3 shows the plasma concentration–time curves of E$_2$-BP and regenerated E$_2$ in rats after intravenous injection of E$_2$-BP, together with those of E$_2$. After its intravenous and oral administration, plasma concentration of E$_2$-BP decreased in a multistep fashion for 28 d. Although regenerated E$_2$ appeared in plasma immediately after injection, its levels were lower than those of E$_2$-BP by a factor of about 1 × 10$^4$ during the initial 4 h. The plasma concentration of regenerated E$_2$ was maintained at the level of less than 1 × 10$^{-4}$% of dose/ml to below the detection limit (1 × 10$^{-6}$% of dose/ml) at 14 d, whereas E$_2$ administered either intravenously or orally showed higher plasma levels compared to regenerated E$_2$ after administration of E$_2$-BP.

**Targeting Characteristics of E$_2$-BP** Table 1 summarizes the AUCs in the bone and plasma after administration of E$_2$-BP and E$_2$, AUC ratios of bone to plasma, and two pharmacokinetic parameters of targeting efficiency, i.e. DTI and TA. The AUC of regenerated E$_2$ in the bone after injection of E$_2$-BP and the AUC of E$_2$ after its intravenous administration was 1.26 and 0.0195, respectively, indicating that the delivery of E$_2$ to the bone was remarkably enhanced by introduction of the bisphosphonic promoiety. TA, which expresses the extent of improvement of the bone delivery, was calculated to be 64.6 on the basis of the AUCs of E$_2$ after intravenous administration of E$_2$-BP and E$_2$. While E$_2$ after oral administration showed smaller AUCs of bone and plasma than those after intravenous injection, either AUC ratio of bone to plasma after intravenous or oral administration of E$_2$ was less than unity. AUC ratios for E$_2$-BP and regenerated E$_2$ after injection of E$_2$-BP, on the other hand, showed very large values of 218 and 169, respectively. From the AUC ratios of bone to plasma after intravenous administration of E$_2$-BP and E$_2$, DTI was calculated to be 451.

**DISCUSSION**

For drugs like estrogen which are rapidly cleared from the body, frequent dosing with conventional dosage forms is required to maintain adequate drug concentration in the target site. This frequent dosing of short half-life drugs results in sharp peak-valley drug concentration–time profiles, and contributes to increased incidence of unwanted side effects. Additionally, the patient receiving estrogen replacement therapy as treatment against postmenopausal osteoporosis should be given the estrogenic products for a long period of time. Consequently, patient compliance in estrogen therapy is often poor. In fact, compliance studies of long-term estrogen therapy have shown that noncompliance stems from adverse effects or the problem of forgetting to take the medication. On the basis of this medical background, we developed E$_2$-BP, the osteotropic prodrug of E$_2$, which functions as not only a site-specific but also a sustained delivery system to the bone.

As shown in Fig. 2, the bone concentration–time profiles of regenerated E$_2$ after intravenous injection of E$_2$-BP clearly indicate that E$_2$-BP achieved the site-specific and sustained delivery of E$_2$ to the bone. The bone concentration of regenerated E$_2$ was maintained for 28 d. E$_2$ after the conventional route of oral administration showed low bone distribution and was rapidly eliminated from the bone. These findings reflect the results of our previous pharmacological studies, where a once per 4 weeks injection of E$_2$-BP increased significantly the bone density com-

![Fig. 3. Plasma Concentration–Time Curves after Administration of E$_2$-BP and E$_2$ in Rats](Image)

Table 1. AUC, AUC Ratio of Bone to Plasma, Drug Targeting Index (DTI) and Therapeutic Availability (TA) in Rats

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Administration route</th>
<th>Compound detected</th>
<th>AUC (% of dose-h/ml or g) Bone</th>
<th>AUC ratio$^a$</th>
<th>TA$^b$</th>
<th>DTI$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_2$-BP</td>
<td>Intravenous</td>
<td>E$_2$-BP</td>
<td>217$^c$</td>
<td>0.996</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>E$_2$-BP</td>
<td>Intravenous</td>
<td>Regenerated E$_2$</td>
<td>1.26$^d$</td>
<td>7.47 × 10$^{-3}$</td>
<td>169</td>
<td>64.6</td>
</tr>
<tr>
<td>E$_2$</td>
<td>Intravenous</td>
<td>E$_2$</td>
<td>19.5 × 10$^{-3}$</td>
<td>51.9 × 10$^{-3}$</td>
<td>0.375</td>
<td>1.00</td>
</tr>
<tr>
<td>E$_2$</td>
<td>Oral</td>
<td>E$_2$</td>
<td>2.56 × 10$^{-3}$</td>
<td>3.46 × 10$^{-3}$</td>
<td>0.740</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ AUC ratio of bone to plasma.  
$^b$ DTI and TA were calculated according to Eqs. 1 and 2 in Materials and Methods, respectively.  
$^c$ Each value represents the mean of five rats.
pared to a daily oral administration of E2 of the same dose. As mentioned, the prolonged bone delivery system of E2 will overcome the problems of poor compliance due to lengthy therapeutic period and adverse effects.

As demonstrated previously,11,12 bone-retention of the parent drug after administration of the bisphosphonate prodruk can be explained by two mechanisms: (i) the diffusion-limited release of the parent drug through the bone mineral matrix, and (ii) the slow penetration of the parent drug through the lining cell,17,18 which separates the bone mineral from extracellular fluid. From a pharmacological point of view, prolonged bone delivery of E2 may restore the bone loss through stimulation of bone formation. Although estrogen is generally considered to maintain bone mass through its antiresorptive action, estrogen reportedly not only reduced bone resorption but also promoted bone formation. E2, delivered continuously to the bone of ovariectomized rats by osmotic minipump increased osteoblast number and osteoid surface.23 Chow et al.19 reported that daily subcutaneous doses of E2, which did not affect its circulating level, stimulated bone formation in female rats. Therefore, E2-BP may not only inhibit bone resorption but also stimulate bone formation.

In general, bisphosphonates are cleared from the bone depending on the bone turnover.20 Several investigators reported that the elimination half-lives of the bisphosphonates from the bone in rats were in the order of months.21-23 The elimination of bisphosphonate prodruk may depend on both hydrolysis of the prodruk and turnover of the bone. In fact, CF-BP, the bisphosphonic prodruk of carboxylfluorescein, was cleared from the bone biexponentially, indicating elimination by hydrolysis of CF-BP bound to mineral component of the bone (t1/2 = 3.2 d) and elimination on the basis of turnover of bone itself (t1/2 = 26.5 d), respectively.12 As to E2-BP, however, there is no evidence of biexponential elimination of regenerated E2 from the bone. Since ovariectomy induces accelerated bone metabolism,24,25 the elimination rate of E2-BP as a result of turnover of bone in ovariectomized rats may differ from that in normal rats.

The plasma concentration of regenerated E2 after injection of E2-BP showed a peak-cut time profile compared with those after intravenous and oral administration. In addition, its level was maintained over 7 d at a concentration of 15—40 pg/ml (1—3 x 10^{-5} % of dose/ml), which is almost equal to the physiological concentration of E2 in rats.26,27 Similarly in ovariectomized rats,1 once per 4 weeks treatment with 0.1 mg/kg E2-BP restored significantly bone mineral reduction with no significant increase in uterine weight, which is used as an index of adverse effects of E2. From these findings, E2-BP would predictably exert preferable pharmacological effects with few adverse effects in clinical use.

We previously showed that ODDS had a potential for improving the potency and therapeutic index of a drug, from the quantitative analysis by means of TA and DTI using a model compound.12 E2-BP also showed excellent TA and DTI (Table 1). In general, a high level of bisphosphonate uptake to the bone depended on the binding to hydroxyapatite in a previous in vitro binding study.13 We obtained similar results in a pilot experiment with E2-BP.20 The large bone AUC value of regenerated E2 is mainly due to predominant uptake of E2-BP into the bone.

As shown in Eq. 1, DTI is defined on the basis of AUC ratios of drug delivered to response and toxicity sites. E2 is widely distributed in the body and caused various undesirable effects. As E2 is substantially lipophilic, its concentration at toxicity sites may immediately reach equilibrium with its plasma concentration.30 Accordingly, we assessed DTI of E2-BP on the assumption that systemic concentration of E2 could be associated with its toxicity. Since bisphosphonates are rapidly cleared from the systemic circulation,17 both E2-BP and regenerated E2 showed extremely large AUC ratios of bone to plasma to give an excellent DTI. These findings suggest that ODDS is an effective approach to enhance the potency and improve the therapeutic index of E2.

In conclusion, pharmacokinetical analysis has revealed the superiority of a bisphosphonic prodruk approach. ODDS can be potentially useful for site-specific and sustained delivery of E2 to the bone. As compared with the conventional dosage form of E2, E2-BP should improve patient compliance in long-term estrogen replacement therapy with improvement in the therapeutic index and less frequent medication.

REFERENCES