Relationship Between Physicochemical and Osteotropic Properties of Bisphosphonic Derivatives: Rational Design for Osteotropic Drug Delivery System (ODDS)

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Purpose. The objective of this investigation is to develop a rational design of Osteotropic Drug Delivery System (ODDS), which we have proposed as a novel method for drug delivery to the skeleton via bisphosphonic prodrug, based on the relationship between physicochemical and pharmacokinetic properties of bisphosphonates.

Methods. The theoretical octanol/water partition coefficients (clog P) of 13 bisphosphonates were calculated by computer software, CLOGP ver. 3.05 (Daylight C.I.S., Inc. Irvine, CA) and related to pharmacokinetic or osteotropic parameters after intravenous injection into rats. On the other hand, to optimize ODDS of diclofenac (DIC-BP), the effects of doses or infusion rates on the *in vivo* disposition were investigated in relation to solubility product value (Ksp) of DIC-BP-calcium complex.

Results. Clog P had good correlations with total plasma clearance, apparent distribution volume and the fraction dose delivered to the whole skeleton after bolus injection into rats ($r = -0.868 \sim -0.914$). The targetability of bisphosphonates to the skeleton was linearly decreased with an increase in clog P value and the more hydrophilic bisphosphonates were suitable for ODDS in bolus administration. On the other hand, DIC-BP, a relatively lipophilic bisphosphonate, was effectively and selectively delivered to the skeleton only when administered as a slow infusion to keep plasma concentration lower than that calculated from Ksp value where DIC-BP could precipitate with calcium in the plasma circulation.

Conclusions. Our results suggest the possibility of a rational design of ODDS via bisphosphonic prodrugs, after consideration of compound lipophilicity and precipitability of bisphosphonate-calcium complex.

KEY WORDS: osteotropic drug delivery system; bisphosphonate; lipophilicity; solubility; distribution volume; plasma clearance.

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ABBREVIATIONS: ODDS, Osteotropic Drug Delivery System; CF-BP, bisphosphonic prodrug of carboxyfluorescein; E_2-BP, bisphosphonic prodrug of 17β-estradiol; DIC-BP, bisphosphonic prodrug of diclofenac; clog P, theoretical calculated octanol/water partition coefficient; Ksp, solubility product; QSAR, quantitative structure-activity relationships; QSPR, quantitative structure-pharmacokinetic relationships

INTRODUCTION

Site-specific drug delivery via a prodrug is expected to be a promising approach to enhance the potency and diminish the side effects of drugs (1–2). Recently, we have proposed a novel method for site-specific and sustained release of drugs to the bone, so-called Osteotropic Drug Delivery System (ODDS), using bisphosphonic prodrugs of carboxyfluorescein (CF-BP), 17β-estradiol (E_2-BP), and diclofenac (DIC-BP) as model drugs (3–8). Bisphosphonates are structurally related to pyrophosphate, which is an endogenous modulator of calcium metabolism (9–12). Because bisphosphonates have a high affinity for bone mineral, they predominantly accumulate in osseous tissues after administration into the body (13–14). In using ODDS, we have taken advantage of this osteotropic property of the bisphosphonates. However, in a series of investigations, we have found that the osteotropic ability differed among bisphosphonates and some bisphosphonic prodrugs such as DIC-BP were only moderately delivered to osseous tissues, but rather accumulated in liver and spleen. Therefore, to understand the compound-to-compound differences of their osteotropic ability is a very significant step in the rational design of ODDS.

Some bisphosphonates are reported to form complexes with solute metals in blood plasma. These complexes are recognized as foreign substances and taken up by the reticuloendotherial systems when they precipitate or form colloids (15–16). With this knowledge, we hypothesized that the precipitate formation of complexes between bisphosphonic prodrug and solute metals in plasma could occur and high precipitability of the complexes was a major factor preventing the skeletal uptake and increasing the hepatosplenic accumulation. In this paper, for 13 types of bisphosphonates including bisphosphonic prodrugs, we examined the relationship between their rat pharmacokinetics after bolus injection and compound lipophilicity as an index of the precipitability of bisphosphonates-metal complexes. Based on our hypothesis, a possible way of maximizing the osteotropic ability and avoiding the hepatosplenic disposition of bisphosphonates could be to keep the plasma concentration lower than that at which the precipitate formation of bisphosphonates and metal occurs via a slow intravenous infusion. This possibility was also investigated in DIC-BP, which highly distributes in liver and spleen after bolus injection at high doses (8). With such results as background, the rational drug design of ODDS will be presented and discussed.

MATERIALS AND METHODS

Compounds

The chemical structures of 13 bisphosphonates are presented in Fig. 1. All bisphosphonates were synthesized at Fujisawa Pharmaceutical Co. Ltd. Eight structurally similar bisphosphonates with different spacers at position X and side chain at position R (Compound 1–8), bisphosphonic prodrugs of carboxyfluorescein with different spacer length of 1, 5, or 10 carbons chain (CF-BP:Compound 9–11), and two types of bisphosphonic prodrugs of pharmacologically active compound, 17β-estradiol (E_2-BP:Compound 12) and diclofenac (DIC-BP:Compound 13), were used. The methods for conju-

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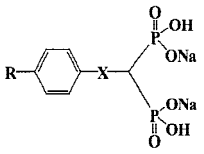
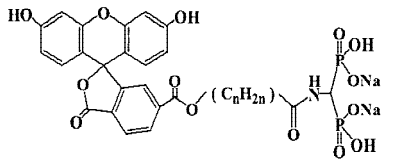
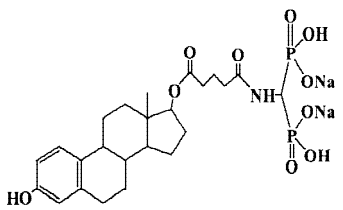


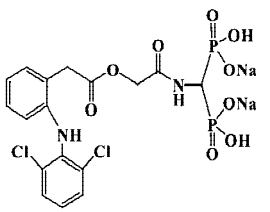
Compound	R	X
1	H	-NH-CS-
2	Cl	-NH-CS-
3	CH_3	-NH-CS-
4	CF_3	-NH-CS-
5	⬡	-NH-CS-
6	H	-NH-CO-NH-
7	H	-CO-NH-
8	H	-SO_2-NH-



9: CF-BP (n = 1) 10: CF-BP (n = 5) 11: CF-BP (n = 10)



12: E_2-BP



13: DIC-BP

Fig. 1. Chemical structures of bisphosphonic derivatives investigated in this study.

gating bisphosphonic moiety with drugs were described elsewhere (3,6,8). All other regents and solvents were of analytical grade.

Calculation of Theoretical Log P

Theoretical calculations of the octanol/water partition coefficient (clog P) of free acids of bisphosphonates were carried out using CLOGP ver. 3.05 (Daylight C.I.S., Inc. Irvine, CA). For all bisphosphonates, the appropriate values of structural fragments were obtained and clog P could be automatically calculated.

Animal Experiments

Sprague-Dawly rats, weighing 190–300 g, purchased from CLEA Japan. Inc. (Tokyo, Japan) were used. All animal studies were performed under the guidelines of the Laboratory Animal Experimental Committee of Fujisawa Pharmaceutical Co., Ltd. As for Compound 1–8, rats intravenously received a single dose of 50 μmole/kg (17.7–21.9 mg/kg) to estimate pharmacokinetic and osteotropic properties. Serial blood samples were withdrawn from the jugular vein at an appropriate interval up to 7 h after dosing and plasma samples were obtained by immediate centrifugation. After the last blood sample was taken, rats were sacrificed to excise femur samples. For Compounds 10–11, at an adequate period after intravenous injection into rats at a dose of 10 μmole/kg (7.1 and 7.8 mg/kg, respectively), rats (four rats per time group) were anesthetized with ether to collect blood samples. Rats were then sacrificed and the femurs removed. As for Compound 9, 12, and 13, pharmacokinetic and osteotropic parameters were quoted or obtained by reanalyzing data from our previous reports (3,7–8).

To elucidate the effects of doses and rates of administration on *in vivo* disposition profile of DIC-BP, another group of rats was included in which femoral vein and bile duct were cannulated. Three rats per group received DIC-BP dissolved in saline from a femoral cannula at a dose of 1 and 10 mg/kg as a bolus injection or constant infusion over 0.25 or 5 h. For rats with bolus administration, blood samples were collected from the jugular vein at 5, 15, and 30 min after injection and plasma samples were obtained by immediate centrifugation to determine the theoretical plasma concentration at time 0 (C_0) as calculated by extrapolation. For rats with constant infusion, blood samples were collected at the end of infusion to determine the maximum plasma concentration of DIC-BP in the experimental period. Twenty-four hours after the bolus injection or completion of infusion, rats were sacrificed and samples of the femur, liver, and spleen were removed. Until the end of experiments, bile and urine samples were collected. All samples were stored in a freezer (–20°C) until assay to avoid post-collection degradation.

Determination Procedures of Bisphosphonates

For analysis of plasma concentrations of Compound 1–8, plasma samples were diluted with distilled water and directly injected into the high-performance liquid chromatography (HPLC) system. To analyze Compound 1–8 in the femur, the femur was dissolved in 6N HCl saturated with NaCl (10% w/v solution). One milliliter of the resultant solution was extracted with 10 ml of acetonitrile. After the organic layer was dried with disodium sulfate anhydride, the organic layer was extracted with 1 M phosphate buffer (pH 7). The aqueous layer was injected into the HPLC system. The HPLC system consisted of a pump unit (Waters Model 510, Waters, Milford, USA), autosampler (Waters WISP 710B, Waters, Milford, USA), and UV detector (Waters Lambda-Max Model 481, Waters, Milford, USA) adjusted to 300 nm for Compound 1–5 or 230 nm for Compound 6–8. The column was reverse phase (Develosil ODS 80™, 5 μm, 100 mm × 4.6 mm, Nomura Kagaku, Aichi, Japan). Flow rate was 1.0 ml/min. The mobile phase was a mixture of 10 mM citrate-phosphate buffer and acetonitrile (~20%) containing tetrabutyl ammonium bromide as ion pair reagent.

The analysis of Compound 9–13 was carried out according to the method of our previous reports (3,7–8).

Data Analysis

To evaluate pharmacokinetic parameters, plasma concentration-time curves for each bisphosphonic derivative

were fitted to the following biexponential equation by nonlinear least-squares method (MULTI) (17):

$$C(t) = A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t)$$

where $C(t)$ represents the plasma concentration at time t. The area under the plasma concentration-time curve (AUC), the total plasma clearance (CL), and the apparent distribution volume in central compartment where bisphosphonic derivatives can instantaneously spread after injection (Vp) were calculated from the following hybrid parameters:

$$AUC = A/\alpha + B/\beta, \quad CL = Dose/AUC, \quad Vp = A + B$$

Scaling up from the amount of femur uptake to that of total body bone was carried out according to the method reported previously (3,8). The equation is as follows;

$$\text{Skeletal distribution (\% of dose/skeleton)} = (\text{\% of dose/g dry femur}) \times (\text{body weight}_{(g)} \times 0.625 \times 10^{-2}) / (103.5 \times 10^{-3})$$

Evaluation of Precipitability of DIC-BP with Calcium Ions in Solution

When DIC-BP and calcium ion are equilibrated with precipitate of DIC-BP-calcium complex in solution, the relationship between the concentrations of DIC-BP and calcium ion is described as;

$$m[\text{DIC-BP}]_{solution} + n\,[Ca^{2+}]_{solution} \Leftrightarrow [\text{DIC–BP–Ca}]_{solid\downarrow}$$

$$[\text{DIC–BP}]_{solution}{}^{m} \times [Ca^{2+}]_{solution}{}^{n} \propto [\text{DIC–BP–Ca}]_{solid}$$

Because $[\text{DIC-BP-Ca}]_{solid}$ can be constant, precipitability of DIC-BP with calcium ions in solution is evaluated with the value of solubility product (Ksp), represented as the minimum formation product of ion concentrations required to induce precipitation.

$$[\text{DIC–BP}]_{solution}{}^{m} \times [Ca^{2+}]_{solution}{}^{n} = K \cdot [\text{DIC–BP–Ca}]_{solid} = \text{constant (Ksp)}$$

where K represents the equilibrate constant of the reaction above.

Firstly, to obtain DIC-BP-calcium complex, 10 mg/ml of DIC-BP solution was added to the solution containing calcium ions and stirred for 3 h to obtain a white suspension of DIC-BP-calcium complex. The resultant precipitate was collected by filtration and washed well with distilled water, and then dried at 40°C under vacuum to obtain a white powder of DIC-BP-calcium complex. We determined a content of calcium in the powder by inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Hitachi, Ibaragi, Japan) and found DIC-BP precipitated with calcium ions in a molecular ratio of 1:1 (i.e. m = n = 1 in the equation above). The resultant powder was suspended again in a distilled solution. After stirring for 24 h (in steady state), the suspension was filtered through a membrane with a pore size of 0.22 μm (Millipore, Tokyo, Japan) and DIC-BP concentration in filtrate was determined. As the concentration of a calcium ion in the solution is equal to that of DIC-BP, Ksp of DIC-BP-calcium complex can be calculated as $[\text{DIC-BP}]^2$.

Table I. Physicochemical and Pharmacokinetic Properties of Bisphosphonates Investigated in This Study[a]

Compound #	M.W. (Dalton)	clog P	Vp (ml)	CL (ml/hr)	Skeletal disposition (% of dose)
1	355.15	–1.184	58.2	77.0	59.4
2	389.60	–0.168	32.7	30.8	65.3
3	369.18	–1.082	40.9	64.7	48.6
4	423.15	0.228	29.9	23.9	50.9
5	437.30	1.436	13.6	7.3	37.3
6	354.10	–1.599	14.5	10.0	52.9
7	353.11	–1.762	55.7	65.4	67.7
8	389.17	–1.721	29.9	48.3	70.2
9	651.32	0.482	22.7	30.3	62.0
10	707.42	1.552	13.8	13.2	35.7
11	777.56	4.197	N.T.[b]	N.T.[b]	6.0
12	603.45	2.147	N.T.[b]	N.T.[b]	11.1
13	571.15	1.790	8.5	4.3	17.9

[a] Pharmacokinetic results are represented as the mean of at least three rats.
[b] Not tested.

RESULTS AND DISCUSSION

Relationship Between Physicochemical and Pharmacokinetic Properties of Bisphosphonic Derivatives Investigated in This Study

Table I summarizes the physicochemical and pharmacokinetic parameters of the bisphosphonates evaluated in this study. The clog P values ranged widely from –1.762 to 4.197. In this study, we focus on the precipitability of bisphosphonate-metal complexes as a major factor affecting the tissue distribution of bisphosphonates. However, it is impossible to theoretically calculate the clog P value of bisphosphonate-metal complexes. Instead, assuming that the mode of complexation with metal ions is common among bisphosphonates, the clog P value of the metal complex would be proportional

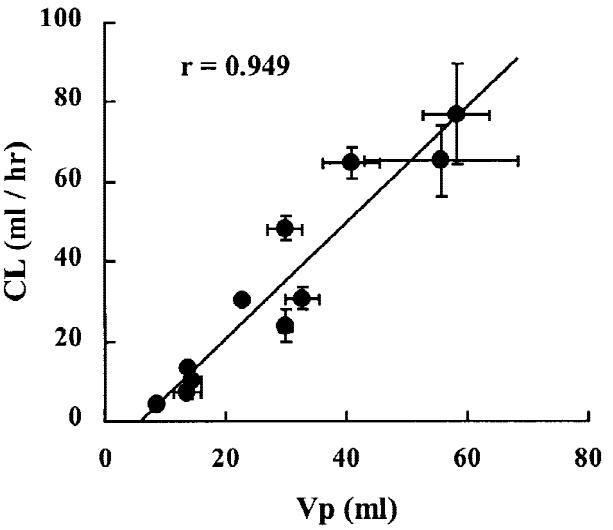


Fig. 2. Correlation of values between distribution volume (Vp) and plasma clearance (CL) after intravenous injection into rats. Results are expressed as the mean ± S.D. of at least three rats. Solid line represents the linear regression result.

to that of a free acid. Thus, we use the clog P value of a free acid as an index of the precipitability of bisphosphonate-metal complexes.

As for pharmacokinetic parameters, the apparent distribution volumes (Vp) ranged from 8.5 ml to 58.2 ml, which are equivalent to rat plasma volume (ca 10 ml) (18) or 6-fold larger at most. In general, Vp is determined by the unbound fraction of the drug in plasma and the affinity to target tissues. Protein binding of some bisphosphonates to rat plasma protein are reported to be high because they can interact with plasma proteins via metal ions such as calcium and ferric ions bound to plasma proteins (19–20). Additionally, precipitate formation with metal ions may partially diminish the unbound concentration of the bisphosphonate in plasma. On the other hand, bisphosphonates can hardly pass the cell membrane due to their physicochemical characteristics of polar and anionic compounds at any biological pH. These properties unique to bisphosphonates may be related to their small Vp values.

Figure 2 shows good correlation between Vp and CL (r = 0.949), suggesting that distribution properties of bisphosphonates have a great influence on their CL values. As shown in Fig. 3, both Vp and CL decreased with increase in clog P. Linear regression analysis for plots of Vp and CL against clog P of bisphosphonates except for Compound 6 obtained good regression coefficients (r = -0.868 and –0.914, respectively). Although the clog P value of Compound 6 is very low, both Vp and CL values were much lower than those of other bisphosphonates (Fig. 3). Hydroxyl or amino group in two side chains in the structure of geminal bisphosphonates is considered to participate in the adsorption to calcium crystal via tridentate binding to crystal surfaces. Probably, the additional amino group in the spacer alters the binding mode and affinity for calcium, which substantially differentiates the pharmacokinetic properties of Compound 6 from other bisphosphonates.

Though all bisphosphonates exhibited high affinity to *in vitro* hydroxyapatite, a major mineral component of skeleton (data not shown), the fraction of skeletal disposition was obviously different among derivatives, ranging from 6.0% of dose for CF-BP with a side chain length of 10 carbons (Compound 11) to 70.2% of dose for Compound 8 (Table I). Figure 4 depicts the relationship between clog P and osteotropic property of bisphosphonates. Compound lipophilicity had a large impact on the pharmacokinetics of bisphosphonates and fraction dose delivered to the skeleton after intravenous injection into rats decreased linearly with an increase in clog P (r = –0.881). Thus, the clog P value can be a predictive index for pharmacokinetic characteristics of bisphosphonates. Our interpretation by deduction is that the precipitability of bisphosphonate-metal complexes could be augmented depending on compound lipophilicity and the extent of precipitate formation, which is subject to phagocytic effects of macrophages and prevents the skeletal uptake of bisphosphonates, could increase.

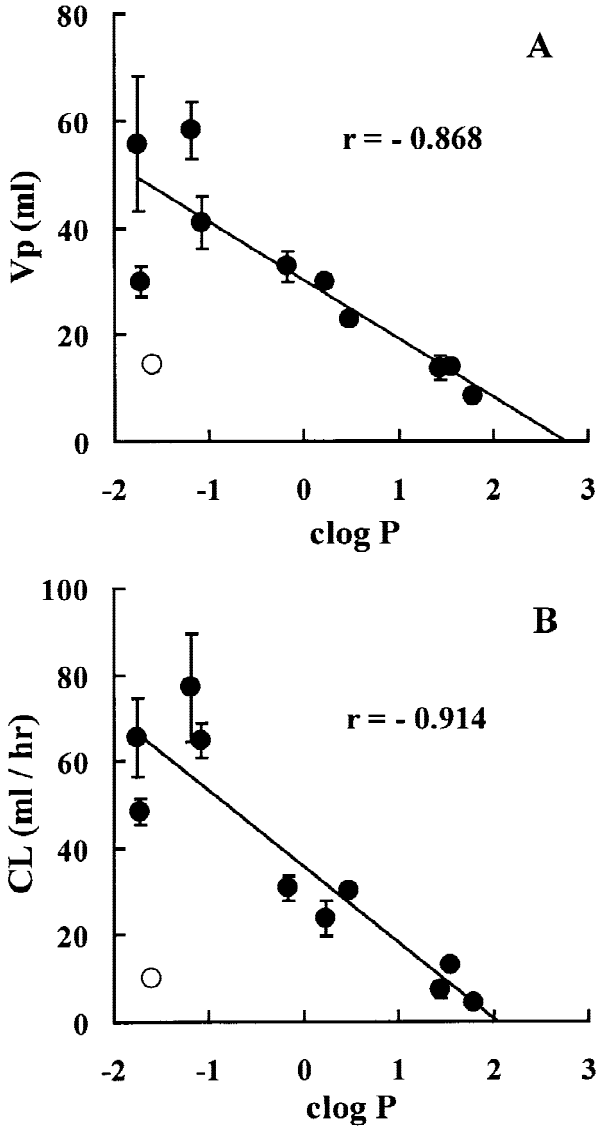


Fig. 3. Correlation of values between lipophilicity (clog P) and (A) distribution volume (Vp) or (B) plasma clearance (CL) after intravenous injection into rats. Results are expressed as the mean ± S.D. of at least three rats. Solid lines represent the linear regression results of plots except for Compound 6 (open symbol).

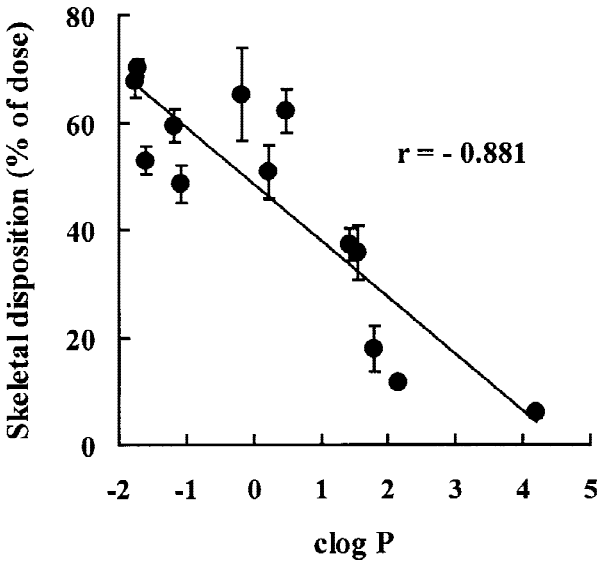


Fig. 4. Correlation of values between lipophilicity (clog P) and amount of recovery in the skeleton after intravenous injection into rats. Results are expressed as the mean ± S.D. of at least three rats. Solid line represents the linear regression result.

Table II. *In Vivo* Disposition Profiles of DIC-BP after Bolus Injection or Constant Infusion into Rats[a]

Dose (mg/kg)	Duration of infusion (hr)	Plasma conc.[b] (μg/ml)	Amount of recovery (% of dose)				
			Skeleton	Liver	Spleen	Urine	Bile
10	bolus	313.2 ± 64.6	24.8 ± 1.4	38.9 ± 1.2	0.9 ± 0.3	3.6 ± 1.8	9.4 ± 0.8
	0.25	241.2 ± 35.3	31.3 ± 3.6	35.0 ± 5.0	0.6 ± 0.2	2.0 ± 1.3	9.6 ± 1.2
	5	64.8 ± 3.7	43.3 ± 4.3	18.4 ± 1.6	0.2 ± 0.0	7.9 ± 0.8	11.7 ± 1.2
1	bolus	19.8 ± 1.7	50.6 ± 6.4	15.0 ± 1.5	0.2 ± 0.2	5.8 ± 1.6	14.2 ± 3.1
	5	0.5 ± 0.1	42.0 ± 3.0	3.3 ± 3.8	n.d.[c]	17.9 ± 1.2	21.9 ± 5.4

[a] Results are represented as the mean ± S.D. of three rats.
[b] Plasma concentration of DIC-BP at time 0 (C_0) calculated by extrapolation after bolus injection or plasma concentration of DIC-BP at the end of constant infusion.
[c] n.d. < about 0.03% of dose.

Effects of Doses or Infusion Rates on *In Vivo* Disposition Characteristics of DIC-BP

We examined whether dose or infusion rate influences *in vivo* disposition characteristics of bisphosphonic derivatives using DIC-BP (Compound 13), which is a relatively lipophilic bisphosphonate and only moderately distributes in osseous tissues (Table I). As shown in Table II, changes in doses and infusion rates altered the maximum plasma concentration of DIC-BP (C_0 value at bolus injection or plasma concentration at the end of infusion) from 0.5 to 313.2 μg/ml. As the maximum plasma concentration decreased, skeletal disposition increased while hepatic and splenic disposition decreased. The fraction doses of urinary and biliary excretion increased as the plasma concentration decreased. As a result, selective drug delivery of DIC-BP to the skeleton was attained after administration at a dose of 1 mg/kg as a 5-h infusion, when maximum plasma concentration was 0.5 μg/ml. On the other hand, the value of solubility product (Ksp) of DIC-BP-calcium complex in aqueous solution was calculated to be 2.0×10^{-8} M^2. Considering the endogenous concentration of calcium ion in rat plasma is reported to be approximately 2×10^{-3} M (21), the minimum plasma concentration of DIC-BP where precipitation of DIC-BP-calcium complex can occur in rat plasma is calculated to be 1×10^{-5} M, corresponding to 5.7 μg/ml. Indeed, only in the case at 1 mg/kg as a 5-h infusion, the plasma concentration of DIC-BP could be maintained much lower than 5.7 μg/ml, where selective delivery to the skeleton with no significant accumulation in the liver or spleen was attained (Table II). Thus, an effective delivery of DIC-BP to the skeleton can be designed by keeping the plasma concentration lower than that at which the precipitation of complex between DIC-BP and calcium occurs.

Quantitative structure-activity relationships (QSAR) relating biological activity to physicochemical properties of drugs have been successfully used for the rational design of new pharmacologically active compounds (22–24). On the other hand, it has also been recognized that pharmacokinetic properties may play a determinant role in drug action and an approach for quantitative structure-pharmacokinetic relationships (QSPR) has been increasingly focused on (25–26). However, little information concerning QSAR and QSPR for bisphosphonates is available except for a few reports (13,27–28). Many clinical results suggest that the rate of infusion has influences biological activity and toxicity of bisphosphonates (29–30). One reason for this is insisted that changing infusion rates alters the extent of the skeletal disposition. Thus, the present investigations on QSPR for bisphosphonates could be of general interests for clinical use. In this paper, we demonstrate that hydrophilic drugs can be effectively and selectively delivered to the skeleton via ODDS with bolus injection. Otherwise, even lipophilic drugs can be applied to ODDS by controlling dose or infusion rate after consideration of precipitability of bisphosphonate-calcium complex. These findings will provide valuable information not only in the development of a rational design and dosage regimen in ODDS, but also to the clinical use of biologically active bisphosphonates.

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