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Mode of administration-dependent pharmacokinetics of bisphosphonates and bioavailability determination

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Abstract

We investigated the influence of mode of administration on the pharmacokinetics of a clinically used bisphosphonate, pamidronate, and of suberoylbisphosphonate (SuBP), a novel bisacylphosphonate of the P–CO–$(C)_n$–CO–P type, in rats. Serum drug levels and tissue disposition were determined following administration of the drugs by different modes: intravenous bolus (iso-osmotic and hypo-osmotic solutions), continuous intravenous infusion, and peroral administration. Results of the study indicate that the disposition of the bisphosphonates in soft tissue (liver, kidney and spleen) was dependent on route and rate of drug administration, and on the osmoticity of the vehicle. Consequently, main pharmacokinetic parameters (AUC, CL, and V_{ss}) were influenced by the mode of drug administration, precluding accurate determination of bioavailability from AUC values. On the other hand, bone and urine bisphosphonate accumulation were considerably less dependent on mode of administration, and, therefore, are recommended for bioavailability calculation.

Keywords: Bisphosphonate; Pharmacokinetics; Bioavailability; Volume of distribution; Mode of administration dependency; Nonlinear pharmacokinetics

1. Introduction

Bisphosphonates are drugs used in various metabolic bone disorders, including hypercalcemia of malignancy, tumor osteolysis, Paget's disease and postmenopausal osteoporosis (Bonjour et al., 1994; Rosen and Kessenich, 1996). Bisphosphonates are characterized by high polarity and high water solubility, resulting in very low oral bioavailability of typically less than 1%. The main drug elimination pathways are kidney excretion and entrapment and accumulation in bone (Lin, 1996; Fleisch, 1997). The bisphosphonates are generally not metabolized and are, therefore, regarded as 'hard drugs' (Lin, 1996; Lin and Lu, 1997).

The clinically used modes of bisphosphonate administration are associated with certain drawbacks and adverse effects. Parenteral routes of administration, such as SC or IM injections, are

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associated with necrosis at the injection site while bolus IV administration may induce acute renal failure especially when a high dose of the drug is rapidly injected (Bounameaux et al., 1983). In addition, accumulation of the drug in soft tissue may occur, which is further augmented when the drug is administered in a hypo-osmotic vehicle (Monkkonen and Ylitalo, 1990). Thus, a slow infusion is required that is inconvenient for the patient. For oral bisphosphonate therapy, a relatively high dose of the drug has to be given to compensate for its poor bioavailability, resulting in a relatively high incidence of adverse GI reactions (Body et al., 1996).

The aims of this investigation were to determine the appropriate approach for estimation of oral bioavailability and to assess the effect of different modes of administration on the distribution pattern of these drugs in different tissues (i.e. biological fluids, soft and calcified tissues). These questions are of particular relevance due to the previous findings that the pharmacokinetics of the bisphosphonates could be nonlinear (Fleisch, 1993; Lin et al., 1994; Lin, 1996; Fleisch, 1997).

To study these questions we assessed the impact of clinically applied modes of administration (Bonjour et al., 1994; Body et al., 1996) on the pharmacokinetics of two bisphosphonates: pamidronate and suberoylbisphosphonate (SuBP) (Fig. 1). Pamidronate, an aminobisphosphonate, is used clinically in Paget's disease and in tumor-induced bone disorders (Bonjour et al., 1994; Rosen and Kessenich, 1996). SuBP is a novel bisacylphosphonate of the P–CO–$(C)_n$–CO–P type that was previously found to have both anticalcification (Golomb et al., 1992b; Van Gelder et al., 1995) and antiresorption activities in animal models (Golomb et al., 1992a; Van Gelder et al., 1995).

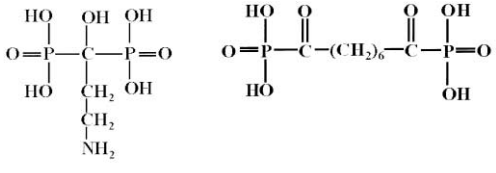

Fig. 1. Structures of bisphosphonates examined in this study.

2. Materials and methods

2.1. Materials

SuBP and [^{14}C]SuBP were synthesized in the laboratory of Dr T. Klenner at the DKFZ, Heidelberg, Germany (Golomb et al., 1992b). Pamidronate, [^{14}C]pamidronate and etidronate were synthesized in the laboratory of Professor E. Breuer at The Hebrew University of Jerusalem. The specific activities of the radiolabeled SuBP and pamidronate were 14.4, and 1 μCi/mg, respectively. All other reagents were of analytical grade.

2.2. Animals

Male Sprague–Dawley rats weighing 215–250 g were kept individually in metabolic cages under 12 h light/dark cycle with free access to water and food for a period of one week prior to the investigation for acclimatization. For pharmacokinetic studies the rats were deprived of food 16 h before the experiment. An indwelling cannula was inserted into the rat's right jugular vein under light ether anesthesia one day before the pharmacokinetic experiment (Hoffman and Levy, 1989). Each experimental group consisted of five to six rats.

2.3. Experimental protocols

2.3.1. Pharmacokinetics and disposition of SuBP and pamidronate

A dose of 1 mg/kg of SuBP or pamidronate was dissolved in iso-osmotic vehicle (0.3 ml normal saline) or hypo-osmotic vehicle (0.3 ml double distilled water, DDW) and administered by IV bolus injection over a 3 min period. A dose of 10 or 40 mg/kg of SuBP or pamidronate was dissolved in 1 ml DDW and administered orally (PO) by a stomach tube. Blood samples (0.3 ml) were collected at 0, 10, 30 min, 1, 2, 3, 5, 6, 8, 24 h post administration. In another experiment 1 mg/kg of SuBP or pamidronate (dissolved in saline) was administered by IV infusion over 4 h (0.5 ml/h). Blood samples were collected at 3 h (for SuBP), at the end of the infusion (for SuBP and pamidronate), and 24 h after the beginning of the infusion (for SuBP and pamidronate). Serum

samples (obtained by immediate centrifugation, 3000 rpm, 10 min) were stored at −20°C pending analysis. The rats were euthanized by overexposure to ether 24 h after drug administration, and the tibia, femur, kidney, liver, intestine, spleen, muscle, brain, urine and feces were obtained and frozen until assay.

The concentrations of the bisphosphonates in serum were determined by combining 0.2 ml serum sample with 10 ml of a liquid scintillation cocktail (Insta-gel ll plus, Packard, Meriden, CT). The radioactivity in rat serum was quantified in a Packard liquid scintillation counter (Packard, Meriden, CT), by using appropriate standard curves. The mean correlation factor, r, was 0.99; and the detection limit was 12 and 15 ng for pamidronate and SuBP, respectively.

The tibia, femur, specimens of the soft tissues (approximately 0.5 g), urine samples (0.5 ml) and feces were combusted in a sample oxidizer (model 307, Packard, Meriden, CT) using Carbo-Sorb carbon dioxide absorber and Permafluor E+ scintillation cocktail (Packard, Meriden, CT). Radioactivity and drug amount (% of dose that was found in the whole organ, urine or feces) were then determined as described above.

2.3.2. Gastric absorption

Gastric absorption of pamidronate was studied in another experimental group. The peritoneal cavity was opened by a midline incision following ketamine and xylazine anesthesia. The pylorus was ligated, the incision was sutured, and the rats were allowed to recover for a period of 2 h. Pamidronate (10 mg/kg) was dissolved in DDW and administered orally (PO) by a stomach tube. Two hours following drug administration the rats were euthanized. The tibia, femur, kidney, liver and spleen were then obtained, and the amount of the drug was determined as described above.

2.3.3. Protein binding

Serum protein binding of SuBP and pamidronate was determined by equilibrium dialysis of human and rat serum at 37°C against an equal volume of 0.13 M sodium and potassium phosphate buffer (pH 7.4). The dialysis was performed for 6 h in Plexiglass cells separated by a cellulose membrane with a molecular exclusion limit of 12000–14000 Da (Visking cellulose tubing, Union Carbide, New York, NY). The free fraction was calculated by the ratio between the amount of the drug in the serum and the buffer solution (Hoffman and Levy, 1989).

2.3.4. Solubility of calcium complexes/salts

The effect of the calcium cation on the solubility of bisphosphonates was determined by mixing 10 ml of 2.5 mM calcium chloride in 0.05M Tris buffer, pH 7.4, with a bisphosphonate (SuBP, pamidronate or etidronate) at concentrations varying from 0.1 to 40 mM. The mixtures were shaken at 37°C for 24 h, and centrifuged after which the calcium concentration in the supernatant was determined by atomic absorption spectroscopy (Golomb and Wagner, 1991).

2.4. Pharmacokinetic analysis

Pharmacokinetic analysis was performed using the WinNonlin program by means of the noncompartmental analysis method. Total clearance was calculated as dose/AUC. The renal clearance (CL_r) and the bisphosphonate uptake by the tibia (CL_{TIBIA}) were calculated as the ratio of the bisphosphonate amount in the organ at 24 h/AUC and were normalized to the weight of the rats. The apparent volume of distribution at steady state (V_{SS}) was calculated as dose· MRT/AUC, where MRT is the mean residence time of the drug in the body (Gibaldi and Perrier, 1982).

In the case of IV infusion (total dose of 1 mg/kg over 4 h), drug steady state serum concentrations were determined at the end of the infusion. The main pharmacokinetic parameters were estimated as follows:

$$CL = k_0/C_{SS}$$

$$AUC = D/CL$$

$$V_{SS} = CL \times MRT_{IV}$$

where CL is total body clearance, k_0 is the rate of drug input, C_{SS} is the steady state concentration; D is the dose; and MRT_{IV} is the mean residence time following the IV iso-osmotic bolus administration.

The bioavailability was determined according to the standard method using AUC data (F_{AUC}) by:

$$F_{AUC} = (AUC_{PO} \cdot D_{IV})/(AUC_{IV} \cdot D_{PO}) \quad (1)$$

Bioavailability values were also calculated using the amounts of drugs, accumulated in bone or urine 24 h following administration, by:

$$F_{TIBIA} = (\text{Amount tibia}_{PO} \cdot D_{IV})/(\text{Amount tibia}_{IV} \cdot D_{PO}) \quad (2)$$

$$F_{FEMUR} = (\text{Amount femur}_{PO} \cdot D_{IV})/(\text{Amount femur}_{IV} \cdot D_{PO}) \quad (3)$$

$$F_{URINE} = (\text{Amount urine}_{PO} \cdot D_{IV})/(\text{Amount urine}_{IV} \cdot D_{PO}) \quad (4)$$

The calculations of absolute bioavailability values were based on IV iso-osmotic bolus data.

2.5. Statistical analysis

The two-tail nonparametric Mann–Whitney *U* test was used for comparison between the groups' values. The Kruskal–Wallis test was used when more than two groups were compared. A *P*-value of less than 0.05 was termed significant.

3. Results

3.1. SuBP pharmacokinetics

The serum SuBP concentration-versus-time profile following a bolus IV administration of 1 mg/kg in iso-osmotic and hypo-osmotic vehicle is presented in Fig. 2. It can be seen that iso-osmotic administration resulted in a rapid elimination rate of SuBP; three hours after administration there were no measurable drug concentrations in the serum. Hypo-osmotic SuBP administration resulted in markedly slower drug elimination from the blood with measurable drug concentrations detected 24 h after the administration. The serum concentrations of SuBP were 588 ± 100 ng/ml at 3 h and 586 ± 101 ng/ml at the end of the IV infusion

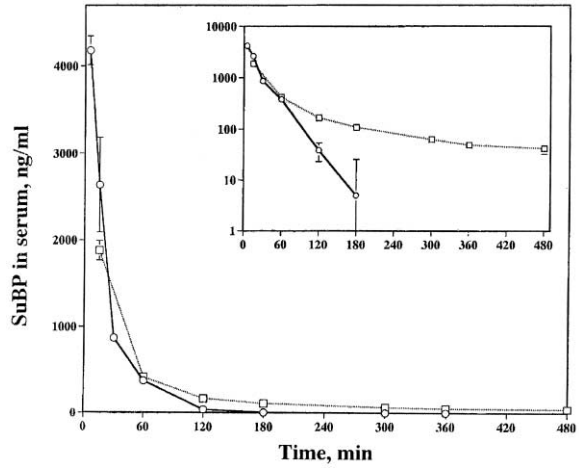

Fig. 2. Mean serum SuBP concentration after administration of 1 mg/kg in iso-osmotic (○) and hypo-osmotic (□) IV bolus modes (mean ± S.D.).

(1 mg/kg over 4 h), indicating that a steady state had been reached. The main pharmacokinetic parameters following different modes of SuBP administration were estimated according to a non-compartmental analysis, and are presented in Table 1.

SuBP serum concentrations versus time following PO administrations of 10 and 40 mg/kg SuBP are plotted in Fig. 3. T_{max} was attained after a short period of time, suggesting rapid (gastric) absorp-

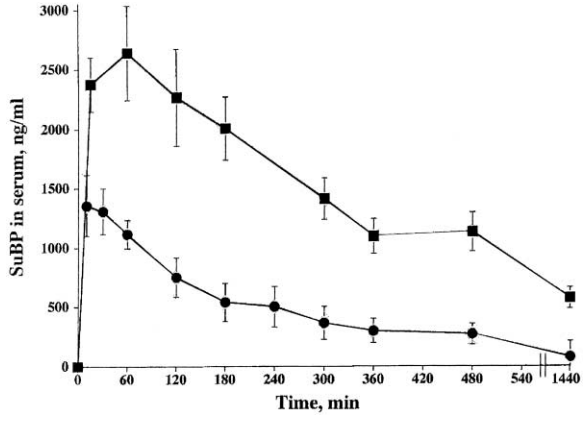

Fig. 3. Mean serum SuBP concentration after 10 mg/kg (●) and 40 mg/kg (■) PO administration (mean ± S.D.).

Table 1
Main pharmacokinetic parameters following SuBP and pamidronate administration (mean ± S.D.)

Drug	Administration	AUC 24h (mcg min/ml)	CL (ml/min per kg)	CL_r (ml/min per kg)	CL_{TIBIA} (ml/min per kg)	$T_{1/2}$ (min)	V_{SS} (ml/kg)
SuBP	IV bolus 1 mg/kg (iso-osmotic)	97.8 ± 21.7	10.6 ± 2.3	5.97 ± 2.59	0.040 ± 0.008	21.7 ± 4.9	307 ± 117
SuBP	IV bolus 1 mg/kg (hypo-osmotic)	160 ± 13	5.88 ± 5.23	1.44 ± 0.95	0.045 ± 0.004	173 ± 51	864 ± 209
SuBP	IV infusion 4h 1 mg/kg	144 ± 21	7.12 ± 1.12	5.74 ± 0.63	0.028 ± 0.005	–	202 ± 32
SuBP	PO 10 mg/kg	428 ± 92	–	1.02 ± 0.59	0.0028 ± 0.0008	288 ± 104	–
SuBP	PO 40 mg/kg	1164 ± 414	–	0.50 ± 0.21	0.0056 ± 0.0023	308 ± 156	–
Kruskal–Wallis test (P)		–	<0.01	<0.01	<0.01	<0.01	<0.05
Pamidronate	IV bolus 1 mg/kg (iso-osmotic)	66.3 ± 7.9	15.2 ± 3.5	1.92 ± 0.75	0.27 ± 0.03	17.2 ± 6.6	183 ± 48
Pamidronate	IV bolus 1 mg/kg (hypo-osmotic)	62.9 ± 12.6	16.4 ± 3.1	1.24 ± 0.41	0.28 ± 0.07	22.2 ± 14.7	273 ± 45
Pamidronate	IV infusion 4h 1 mg/kg	185 ± 19	5.45 ± 0.53	0.37 ± 0.18	0.081 ± 0.022	–	64.8 ± 6.6
Pamidronate	PO 10 mg/kg	43.0 ± 21.2	–	0.43 ± 0.21	0.011 ± 0.006	105 ± 38	–
Kruskal–Wallis test (P)		–	<0.01	<0.05	<0.01	<0.01	<0.01

Table 2
Disposition of SuBP in various organs 24 h after drug administration (% of administered dose ± S.D.)[a]

Administration	Urine	Feces	Tibia	Femur	Kidney	Liver	Intestine	Spleen
IV bolus 1 mg/kg (iso-osmotic)	55.8 ± 20.0	2.57 ± 1.15	0.38 ± 0.02	0.47 ± 0.04	0.19 ± 0.04	0.20 ± 0.04	ND	ND
IV bolus 1 mg/kg (hypo-osmotic)	24.2 ± 14.7[b]	1.31 ± 0.47	0.79 ± 0.02[b]	0.70 ± 0.08	0.84 ± 0.03[b]	0.57 ± 0.03[b]	0.091 ± 0.016	–
IV infusion 4h 1 mg/kg	81.2 ± 7.2	1.02 ± 0.29	0.40 ± 0.03	0.48 ± 0.05	0.18 ± 0.01	0.28 ± 0.03	0.21 ± 0.05	ND
PO 10 mg/kg	3.81 ± 1.05	89.2 ± 13.8	0.012 ± 0.001	0.019 ± 0.002	0.017 ± 0.001	0.168 ± 0.016	0.087 ± 0.016	ND
PO 40 mg/kg	1.45 ± 0.30	103 ± 9.83	0.017 ± 0.003	0.019 ± 0.005	0.027 ± 0.004	0.079 ± 0.017	0.385 ± 0.166	–

[a] ND, not detected; –, not determined.
[b] Significantly different in comparison to iso-osmotic IV bolus ($P<0.05$).

tion of the drug. The longer elimination half lives following oral in comparison to bolus administrations (Table 1) indicates that SuBP exhibited flip-flop kinetics.

Tissue distribution data of SuBP following IV and PO administration are summarized in Table 2. Approximately 56% of the dose was detected in the urine 24 h after IV iso-osmotic administration in contrast to exceedingly low SuBP amounts in the soft tissues and feces. SuBP tissue distribution following administration of iso-osmotic IV bolus dose and IV infusion was similar, apart from a slightly higher disposition in the liver following the infusion mode (Table 2). SuBP IV administration in hypo-osmotic vehicle resulted in a significantly different drug tissue distribution profile than that observed following administration of the same dose in iso-osmotic vehicle. Statistically significant higher concentrations of SuBP were found in the bones, kidney and liver, with a lower disposition in urine.

When SuBP was given orally most of the drug was detected in the feces, whereas exceedingly low concentrations were measured in soft tissues and in urine (Table 2). The bioavailability of SuBP 10 mg/kg, F_{AUC}, was found to be 38.9%. In contrast, F_{URINE}, F_{FEMUR} and F_{TIBIA} were 6.8, 3.2 and 4.0%, respectively.

3.2. Pamidronate pharmacokinetics

Serum pamidronate concentrations following both IV bolus administrations of iso-osmotic and hypo-osmotic solutions were found to be nearly identical (see Fig. 4). In both cases the drug serum concentration declined rapidly, and practically no drug was detected 1.5 h after administration. The steady state serum concentration of pamidronate following constant IV infusion (1 mg/kg over 4 h) was 770 ± 79 ng/ml. The elimination rate of pamidronate after IV bolus administration was

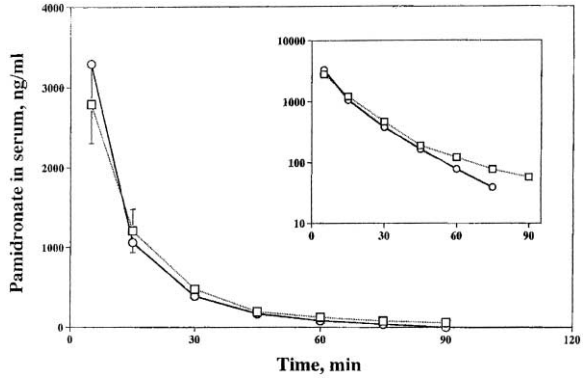

Fig. 4. Mean serum pamidronate concentration after administration of 1 mg/kg in iso-osmotic (○) and hypo-osmotic (□) IV bolus modes (mean ± S.D.).

Table 3
Disposition of pamidronate in various organs 24 h after drug administration (% of administered dose ± S.D.)[a]

Administration	Urine	Feces	Tibia	Femur	Kidney	Liver	Intestine	Spleen
IV bolus 1 mg/kg (iso-osmotic)	12.10 ± 4.27	0.29 ± 0.30	1.69 ± 0.19	2.22 ± 0.22	0.083 ± 0.012	4.50 ± 1.61	ND	0.200 ± 0.052
IV bolus 1 mg/kg (hypo-osmotic)	7.08 ± 7.42	0.51 ± 0.42	1.73 ± 0.12	2.29 ± 0.13	0.105 ± 0.012[b]	9.60 ± 1.77	ND	0.644 ± 0.092
IV infusion 4h 1 mg/kg	6.01 ± 3.04[b]	0.58 ± 0.37	1.48 ± 0.24	2.14 ± 0.21	0.094 ± 0.013	1.02 ± 0.28[c]	ND	0.096 ± 0.038[c]
PO 10 mg/kg	0.119 ± 0.090	87.1 ± 18.0	0.0066 ± 0.0049	0.0086 ± 0.0050	0.0013 ± 0.0003	0.024 ± 0.008	–	0.0022 ± 0.0018
PO 10 mg/kg pyloric ligation	–	–	0.0060 ± 0.0007	0.0081 ± 0.0005	0.0047 ± 0.0008	0.039 ± 0.007	–	0.0037 ± 0.0005
PO 40 mg/kg	0.078 ± 0.008	81.4 ± 4.0	0.0076 ± 0.0019	0.0087 ± 0.0011	0.0033 ± 0.0004	0.015 ± 0.0018	0.033 ± 0.005	–

[a] ND, not detected; –, not determined.
[b] Significantly different in comparison to iso-osmotic IV bolus ($P<0.05$).
[c] Significantly different in comparison to hypo-osmotic IV bolus ($P<0.05$).

rapid (a half life of approximately 20 min), indicating that infusion over 4 h can be regarded as steady state condition (i.e. $T>4\times t_{1/2}$). Low pamidronate serum concentrations were found following 10 mg/kg PO administration, but measurable concentrations of the drug were found in all blood samples obtained over a 24-h period. Main pharmacokinetic parameters following pamidronate administration are presented in Table 1.

The tissue distribution of pamidronate following various routes of drug administration is presented in Table 3. Insignificant differences of bone pamidronate concentrations were found among the three modes of drug administration (i.e. iso-osmotic, hypo-osmotic bolus and infusion). In contrast, soft tissue concentrations were found to be dependent on the mode of administration. For example, drug amounts in the liver and in the spleen were found to be significantly lower following continuous infusion of the drug in comparison to bolus administration of the same dose. The iso-osmotic IV bolus administration resulted in markedly lower disposition in the liver and in the spleen than that observed following hypo-osmotic mode of administration. The amount of pamidronate in urine was lower than expected possibly due to incomplete sample collection (as a result of drug sedimentation in the urine collecting tubes).

PO pamidronate administration resulted in low drug absorption, as evidenced by low amounts of drug found in bone, tissue and urine as detected 24 h following administration, whereas a high portion of the dose was found to be excreted in the feces (Table 3). Flip-flop kinetics was observed, as evidenced by the longer elimination half-life found following 10 mg/kg PO dose in comparison to IV bolus administration (Table 1). Most of the pamidronate that reached the systemic circulation following PO administration was absorbed in the upper parts of the gastrointestinal system. When the pylorus was ligated, the amount of drug found in bone following 2 h of gastric absorption was only 20–25% less than that detected in the bone 24 h after PO administration of the same dose to intact animals (Table 3).

The bioavailability of pamidronate (F_{AUC}) fol-

lowing 10 mg/kg PO administration was calculated to be 6.8%. In contrast, the bioavailability calculated according to drug accumulation in bone and in urine: F_{FEMUR}, F_{TIBIA} and F_{URINE}, was 0.39, 0.39 and 1.0%, respectively.

3.3. Protein binding and solubility

The degree of SuBP binding to rat and human serum protein at different drug concentrations is presented in Fig. 5. Nonlinearity in SuBP protein binding was observed, as evidenced by the reduced degree of drug binding to serum proteins at higher concentrations. On the other hand, pamidronate protein binding was relatively constant (approximately 92%) in the range 0.2–10 g/ml but decreased at higher concentrations (graph not shown).

The effect of calcium (in physiological concentrations of 2.5 mM) on the solubility of SuBP and the geminal bisphosphonates (pamidronate and etidronate) in various concentrations is depicted in Fig. 6. It can be seen that a pronounced difference exists between the solubility of the two types of bisphosphonates. SuBP solubility was not affected by calcium even at high concentrations (35 mM). On the other hand, the solubility of pamidronate and etidronate was markedly reduced even at bisphosphonate concentrations as low as 0.5 mM.

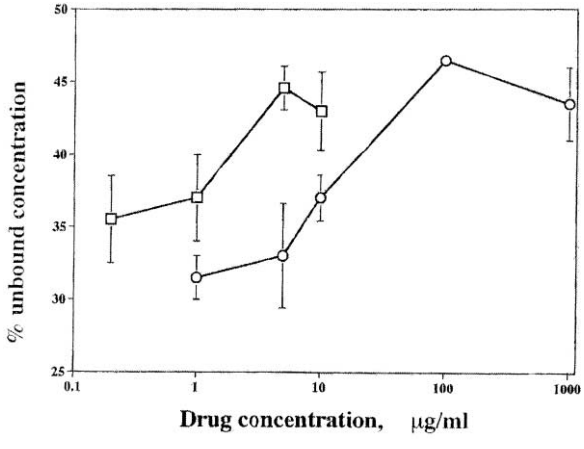

Fig. 5. Percent of unbound SuBP in human (□) and rat (○) serum, determined in dialysis chambers (mean ± S.D.).

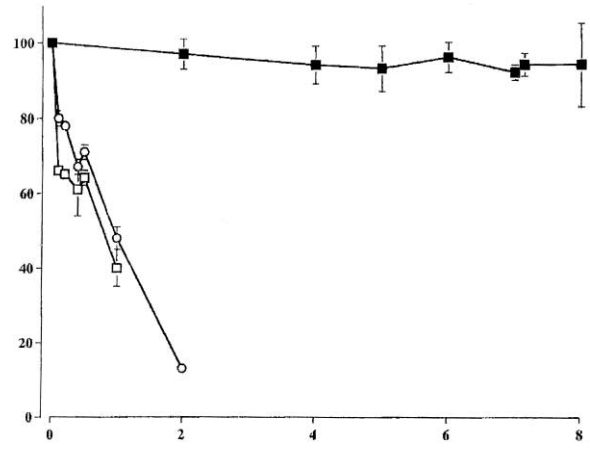

Fig. 6. The solubility of pamidronate (○), etidronate (□) and SuBP (■) at different concentrations of the bisphosphonates in a constant 2.5 mM calcium chloride solution at pH 7.4 ($n = 4$).

4. Discussion

The bisphosphonates have unique pharmacokinetic characteristics that result from their highly polar chemical structure. They lack the usual substrate properties that would make them susceptible to metabolizing enzymes, and thus are not metabolized in the body (Lin and Lu, 1997). By Ariens' definition, bisphosphonates can be categorized as 'hard drugs'. While there is direct proof that pamidronate is not metabolized in laboratory animals (Michael et al., 1972; Wingen and Schmahl, 1987), we assumed that the new bisphosphonate SuBP follows the same trend due to the similarity (i.e. two phosphonate groups) in its molecular structure.

4.1. Determination of bisphosphonate oral bioavailability

The main elimination pathways of the bisphosphonates are kidney excretion, and entrapment

and accumulation in bone (Lin, 1996; Fleisch, 1997). The bone uptake of these drugs is considered to be an irreversible loss process definable in clearance terms analogous to renal clearance (Lin et al., 1994). Therefore, it has been common practice to determine the bisphosphonate bioavailability from the amount of drug retained in bone or in urine (Lin, 1996).

In this study significant differences were found between oral bioavailability values calculated by the standard AUC method (F_{AUC}) and those determined by the amount of drug accumulated in the urine and bones. The same tendency was found for the new bisphosphonate SuBP and for the known compound pamidronate. Based on F_{URINE} and F_{BONE} (i.e. F_{TIBIA} and F_{FEMUR}), the oral bioavailability of SuBP 10 mg/kg was 3–7%, whereas the F_{AUC} value was 38.9% (5–10-fold higher); similarly, for pamidronate F_{URINE} and F_{BONE} for PO 10 mg/kg administration were less than 1%, whereas F_{AUC} was 6.8%. Had there been linear pharmacokinetics F_{AUC}, F_{URINE} and F_{BONE} should have been equal.

We think that F_{AUC} values are inappropriately high and are derived from the effect of the mode of administration on the concentration–time data. On the other hand, F_{URINE} and F_{BONE} are less influenced by temporal changes, thus providing a better estimation of oral bioavailability. This conclusion is in accord with previous reports, based on mass-balance calculations, which showed very low oral bioavailability ($F < 1\%$) for pamidronate (Wingen and Schmahl, 1987; Hyldstrup et al., 1993), similar to the values of F_{URINE} and F_{BONE} found in this work. So far, the inappropriateness of using AUC for determination of bisphosphonate oral bioavailability has not been highlighted due to the analytical limitations of quantifying the relatively low blood concentrations (Lin, 1996). In addition to the direct influence on F_{AUC}, disproportionate AUC values also affected the values of both CL_{TIBIA} and CL_r. As shown in Table 1, CL_r and CL_{TIBIA} (which should be constant in linear pharmacokinetics) varied profoundly following different modes of administration. Since these clearance values are determined as the ratio between the amount of drug accumulated in the urine or the tibia over 24 h and AUC_{24h}, their value is directly affected by the concentration–time data of the bisphosphonate.

4.2. Mode of administration-dependency

Following IV administration of the same dose (1 mg/kg) by different modes (i.e. iso-osmotic and hypo-osmotic IV bolus, slow IV infusion), different values of AUC were obtained, with lower values for the bolus administration (Table 1). This mode of administration-dependency is attributed to nonlinear tissue distribution (i.e. liver, kidney spleen) of the bisphosphonates (see Tables 2 and 3). The lower AUC values detected following bolus administration could result from temporally elevated clearance because of the relatively high blood concentrations of the drug immediately following bolus administration in comparison to continuous infusion. Such elevation in clearance could result from several mechanisms including entrapment of the drug in various tissues or reduced binding to serum proteins (i.e. saturable binding process).

Lower amounts of pamidronate in soft tissue were observed following IV infusion in comparison to bolus administration (see Table 3). This is due to the low solubility of pamidronate in the presence of cations (Fig. 5). Elevated serum concentrations of the drug are known to form increasing amounts of insoluble pamidronate–cation complexes (Kautiainen et al., 1998) that are taken up by reticuloendothelial tissues (i.e. liver and spleen) (Monkkonen and Ylitalo, 1990). The soft tissue disposition was further increased following the drug's administration in a hypo-osmotic vehicle due to confined hemolysis and to the formation of insoluble pamidronate–cation complexes with cell debris (Monkkonen and Ylitalo, 1990; Osterman et al., 1994).

In contrast to pamidronate, SuBP is soluble in the presence of cations (see Fig. 5) and, therefore, accumulated to the same extent in soft tissues following administration of both iso-osmotic IV bolus and IV infusion. However, unlike pamidronate, the serum protein binding of SuBP is saturable (in the relevant concentrations range,

see Fig. 5) and thereby produces higher unbound fractions and a larger volume of distribution at high concentrations. In this case the apparent clearance of the drug, which is influenced by the total drug concentration in the blood, varies as a function of the mode of administration, while the intrinsic clearance remains constant. This is in accord with the similarity in the total amounts of the drug detected in urine and bone respectively following the two modes of intravenous administration. The reduced renal elimination following bolus administration of SuBP in hypo-osmotic solution indicates that this drug could be nephrotoxic at elevated concentrations (see Table 2), a conclusion that has to be further evaluated.

Administration of both pamidronate and SuBP by the PO route resulted in considerably higher ratios of drug amounts retained in noncalcified/calcified tissues compared to IV infusion (see Table 4). This outcome could be attributed to the two main differences in drug input function between the continuous infusion and the oral administration: (A) Duration of drug input that was only 4 h for the infusion and lasted much longer following PO administration (with apparent absorption rate-constant of 0.14/h and 0.4/h for SuBP and pamidronate, respectively). Thus, the period of time where only elimination processes occurred was 20 h for the infusion but considerably less for the PO administration, which explains the higher drug concentrations found in the liver and kidney at the sampling time (24 h after onset of administration). (B) Unlike the slow elevation in drug concentration following continuous infusion, PO administration produced a high peak serum concentration shortly after the administration (see Fig. 3), suggesting extensive absorption from the stomach and upper intestine. This conclusion is supported by the results of the pyloric ligation experiment, where pamidronate bone accumulation was almost completed (75–80% of the amount accumulated in bone 24 h after ordinary PO administration, see Table 3) 2 h after administration, when only the gastric absorption route was available. The high initial peak concentration could lead to increased entrapment of the drug (particularly pamidronate) in soft tissues, as discussed above for bolus administration.

5. Conclusions

The outcomes of the present investigation indicate that the distribution profile of bisphosphonates in soft tissue and volume of distribution are mode of administration-dependent. The non-linear disposition, particularly at high concentrations, yielded unreliable AUC values that led to inappropriately high F_{AUC} values. Thus, this work provides another reason that AUC method should not be used for these drugs. This is in addition to the practical obstacles that evolve from an analytical limitation of quantifying low blood bisphosphonate concentrations. On the other hand, bioavailability estimation from the amounts of bisphosphonate in bone and urine 24 h after the administration was practically independent of mode of administration. Therefore, the results justify the common practice to determine bisphosphonate bioavailability in pre-clinical studies according to the amount of drug retained in bone, and in clinical experiments according to the amount of drug in urine.

Table 4
Amounts of Drug Disposed in the Liver and Kidney (normalized to the amounts disposed in bone)

SuBP		Pamidronate		*Drug*
Liver/tibia	kidney/tibia	liver/tibia	Kidney/tibia	*Ratio*
0.85 ± 0.28	0.51 ± 0.05	0.70 ± 0.21	0.064 ± 0.007	IV infusion 1 mg/kg
15.0 ± 4.4	1.54 ± 0.56	6.59 ± 7.17	0.33 ± 0.29	10 mg/kg PO
6.7 ± 5.3	2.52 ± 2.36	2.79 ± 1.75	0.65 ± 0.55	40 mg/kg PO
$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	*Kruskal-Wallis test*

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