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DEVELOPMENT OF SUBCUTANEOUS AND INTRAMUSCULAR FORMULATIONS OF CALCIUM ALENDRONATE SALTS

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ABSTRACT

Poorly soluble calcium-alendronate salts were prepared and investigated as potential candidates for subcutaneous or intramuscular formulations. Three such formulations containing calcium-alendronate salts with different stoichiometries were developed for testing in safety, disposition and efficacy studies in animals. All formulations demonstrated a drastic reduction in pain on injection and tissue damaging propensity compared to the soluble salts of ABP. All three were efficacious and showed prolonged absorption from the injection site with the deposition of a large percentage of the dose into the bone. Complex formation between alendronate and calcium was also studied.

INTRODUCTION

Alendronate (4-amino-1-hydroxybutane-1,1-bisphosphonic acid, ABP) is a drug with utility in treatment of diseases characterized by abnormal bone turnover, such as metastatic bone disease, hypercalcemia of malignancy, Paget's disease, and osteoporosis. Parenterally, ABP is given by slow intravenous infusion, which is considered inconvenient in outpatient situations. An obvious choice

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for an alternative formulation is a suitable subcutaneous (SC) or intramuscular (IM) injection. However, soluble forms of ABP, like those of other bisphosphonates, are known to cause pain, irritation and local tissue damage following subcutaneous or intramuscular injection.[1] Thus, the need existed to develop an efficacious SC/IM formulation of ABP with decreased propensity for tissue damage. This formulation should also act as a slow release formula in order to maximize disposition of the drug to the bone. It was established that buffered solutions of soluble ABP sodium salts at a concentration of less than 1 mg/ml did not cause pain in acute pain on injection screening studies. The goal of this study was to identify suitable, poorly soluble forms of the drug and formulate them as stable suspensions. In this paper we describe the work that resulted in development of three such formulations based on calcium salts of ABP.

MATERIALS AND METHODS

Materials - 4-amino-1-hydroxybutane-1,1-bisphosphonic acid sodium salt trihydrate ($ABPNa{\cdot}3H_2O$) was prepared by Merck Research Laboratories Process Research Department and used as received. All other materials and standard solutions were purchased from Aldrich or Fisher Scientific and used as received.

Preparation of $(ABP)_2Ca$ Crystalline Salt - $ABPNa{\cdot}3H_2O$, 3.25 g (10 mmole), was dissolved with heating (~80°C) in 100 ml of 0.01 M HCl. $CaCl_2$, 5 ml of 1.0 M solution (5 mmole), was added to this solution with stirring. The crystallization commenced after a short lag period at which time the heating was discontinued and the mixture was allowed to slowly cool down to room temperature. After the crystallization was complete the crystalline ABP_2Ca was collected by filtration, washed with small amount of cold water and air-dried for several hours. The yield of crystalline ABP_2Ca was usually >80%.[2] The stoichiometry of the salt was confirmed by elemental analysis; % calculated for $C_8H_{24}N_2O_{14}P_4Ca$ (M.W. 536.26): C 17.92, H 4.51, N 5.22, P 23.10, Ca, 7.47; % found: C 17.83, H 4.43, N 5.15, P 22.98, Ca 7.40. The crystallinity of ABP_2Ca was confirmed by optical microscopy and x-ray powder diffraction.

Preparation of $ABPCa{\cdot}H_2O$ Crystalline Salt - A solution of $ABPNa_2$ was prepared by dissolving 3.25 g (10 mmole) of $ABPNa{\cdot}3H_2O$ in 50 ml of 0.1 M NaOH (10 mmole of HO^-). $CaCl_2$, 10 ml of 1.0 M solution (10

mmole of Ca^{2+}), was added to this solution with stirring. Upon addition of the $CaCl_2$ solution heavy precipitation of an amorphous $ABPCa_x$ salt was observed (*vide infra*). The heating of the slurry at ~90°C for 2 hours resulted in complete conversion to crystalline $ABPCa \cdot H_2O$. At this time heating was discontinued and the mixture was allowed to slowly cool to room temperature. Crystalline $ABPCa \cdot H_2O$ was collected by filtration, washed with a small amount of cold water and air-dried for 24 hours. The yield of crystalline $ABPCa \cdot H_2O$ was usually ~90%.[2] The stoichiometry of the salt was confirmed by elemental analysis; % calculated for $C_4H_{11}NO_7P_2Ca \cdot H_2O$ (M.W. 305.18): C 15.74, H 4.29, N 4.59, P 20.30, Ca 13.13; % found: C 15.79, H 4.14, N 4.52, P 20.23, Ca 13.30. Thermogravimetric analysis showed a defined loss of 6.17% weight between 100 and 175°C (5.90% calculated for 1 mole of H_2O) indicating that the material was a stoichiometric monohydrate. The crystallinity of $ABPCa \cdot H_2O$ was confirmed by optical microscopy and x-ray powder diffraction.

Preparation of $ABPCa_x$ Amorphous Salts - Amorphous $ABPCa_x$ salts were precipitated by addition of a $CaCl_2$ solution to solutions of ABP in which the degree of neutralization of ABP was varied in a systematic way (*vide infra*). Since $ABPCa_x$ salts initially precipitated as very fine floculant suspensions they were not routinely isolated as solids. Instead, the emphasis was placed on identifying the conditions under which the most physically stable suspension with satisfactory pH and ABP solubility was formed.[2] In several instances the solid material from the optimized suspensions was isolated for analytical purposes. An example follows: $ABPNa \cdot 3H_2O$, 1.31 g (4.0 mmole), was dissolved in a mixture of 25 ml of water and 7.23 ml of 1.0 M NaOH (7.23 mmole). The $ABPCa_x$ salt was precipitated by addition of 56.2 ml of 0.1 M $CaCl_2$ solution (5.62 mmole). The volume was made up to 100 ml to give a 10 mg/ml suspension expressed as ABP free-acid. The molar ratio of components in this suspension was: $ABP^{n-} : Na^+ : Ca^{2+} = 1 : 2.8 : 1.4$. The resulting $ABPCa_x$ salt was isolated by filtration, washed with a small amount of water and dried for 24 hours at 80°C. The composition of the material was determined by elemental analysis: found weight %: C 13.73, H 3.97, N 4.13; and ICP analysis for Ca^{2+} content: 15.82 weight %. The water content determined by weight loss on heating (TGA) was 8.6 weight %. The above results are consistent with $(ABP)_5Ca_7(H_2O)_8(OH)_4$ as the composition of the isolated amorphous salt.

Methods - X-ray diffraction patterns were obtained using Siemens D5000 x-ray diffractometer. Thermogravimetric analysis was performed using Perkin-Elmer TGA 7 thermogravimetric analyzer. Elemental analysis was performed by Merck Research Laboratories Microanalysis Laboratory. Concentrations of ABP in solution were determined by HPLC.[3] Total concentrations of Ca^{2+} in solution were determined using a Perkin-Elmer P-40 Inductively Coupled Plasma Atomic Emission Spectrometer (ICP). Concentrations of free (uncomplexed) Ca^{2+} in solution were determined using Orion Research microprocessor ionalyzer/901 equipped with Orion Research Ca^{2+} selective liquid membrane electrode coupled with Orion Research Ag/AgCl reference electrode. The Ca^{2+} selective electrode was calibrated with Orion Research $CaCl_2$ standard solutions prior to each measurement. pH measurements were performed using Orion Research microprocessor ionalyzer/901 equipped with Fisher Scientific glass / Ag/AgCl combination electrode.

RESULTS AND DISCUSSION

Calcium Binding Constants of ABP - Titrations of ABP with Ca^{2+} were performed at 25°C at pH 4.2 (native pH of a saturated solution of $ABPNa{\cdot}3H_2O$) and pH 7.5 (physiological pH) in order to establish the ABP-Ca^{2+} equilibrium binding constants. At pH 4.2 the data can be accommodated using equations 1 and 2.

$$ABP^- + Ca^{2+} \underset{}{\overset{K_1}{\rightleftharpoons}} (ABPCa)^+ \qquad (1)$$

$$(ABPCa)^+ + ABP^- \overset{K_2}{\rightleftharpoons} (ABP)_2Ca \qquad (2)$$

Equations 3 and 4 define the equilibrium constants given in equations 1 and 2. Equations 5 and 6 give the concentrations of the two ABP-Ca complexes. Equations 7 and 8 define the concentration of uncomplexed ABP as a function of the total concentration of ABP and the concentration of uncomplexed Ca^{2+}. Substitution of equations 5 and 6 into equation 9 give equation 10. When equation 8 is substituted into equation 10, the total concentration of Ca^{2+} becomes the function of two equilibrium constants (K_1 and K_2), a known total concentration of ABP and the concentration of uncomplexed free Ca^{2+} which is determined experimentally using a Ca^{2+} selective electrode.

$$K_1 = [ABPCa] / ([ABP]_{free}\,[Ca]_{free}) \qquad (3)$$

$$K_2 = [ABP_2Ca] / (K_1 [ABP]_{free}^2 [Ca]_{free}) \quad (4)$$

$$[ABPCa] = K_1 [ABP]_{free} [Ca]_{free} \quad (5)$$

$$[ABP_2Ca] = K_1 K_2 [ABP]_{free}^2 [Ca]_{free} \quad (6)$$

$$[ABP]_{free} = [ABP]_{total} - [ABPCa] - 2[ABP_2Ca] \quad (7)$$

$$[ABP]_{free} = (-K_1[Ca]_{free} - 1 + sqrt((K_1[Ca]_{free})^2 + 8K_1K_2[ABP]_{total}[Ca]_{free}))/(4K_1K_2[Ca]_{free}) \quad (8)$$

$$[Ca]_{total} = [Ca]_{free} + [ABPCa] + [ABP_2Ca] \quad (9)$$

$$[Ca]_{total} = [Ca]_{free}(1 + K_1[ABP]_{free} + K_1K_2[ABP]_{free}^2) \quad (10)$$

Figure 1 shows the titration curves of ABP with Ca^{2+} at four different total concentrations of ABP. The non-linear least square fitting of the titration data to equation 10 gave the following values for the Ca^{2+} binding equilibrium constants: $K_1 = 84 \pm 13\ M^{-1}$, and $K_2 = 33 \pm 8\ M^{-1}$. The good fit of the experimental data to equation 10 implies that at pH 4.2 a stoichiometric complexation occurs between ABP and Ca^{2+} producing $(ABPCa)^+$ and ABP_2Ca complexes.

At pH 7.5 the titration curves could not be fitted to any simple model. This is indicative of the formation of non-stoichiometric polynuclear complexes ($ABPCa_x$) between ABP and Ca^{2+}. The formation of polynuclear complexes was studied extensively in the case of 1-hydroxyethane-1,1-bisphosphonic acid (EDPH) and Ca^{2+} in the physiological pH range. Poor solubility of $ABPCa_x$ complexes further complicated detailed complexation studies at pH 7.5. Comparison of the formation numbers, $Z = ([Ca^{2+}]_{total} - [Ca^{2+}]_{free})/[ABP]_{total}$, shows that the binding between ABP and Ca^{2+} is roughly 10 fold tighter at higher pH (Figure 2). We also have shown that $ABPCa_{1.35}$ best describes the stoichiometry of the precipitates in equilibrium with solutions of $ABPCa_x$ polynuclear complexes (*vide infra*). The extent of calcium binding to ABP was an important factor during formulation development since calcium depletion from the tissue at the injection site may contribute to local irritation.

Preparation of ABP_2Ca Salt and its Suspensions - Crystalline ABP_2Ca is formed according to equation 11.

$$2\ ABP^- + Ca^{2+} \xrightarrow{pH = 2\text{-}2.5} ABP_2Ca(s) \quad (11)$$

When ABP_2Ca was suspended in water or normal saline the pH rapidly dropped to the equilibrium value of 4.7. This pH is not

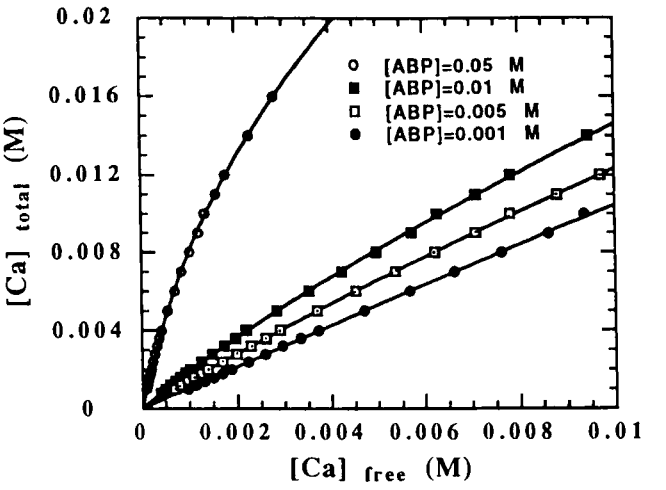

FIGURE 1

Titration curves of ABP with Ca^{2+} at 25°C and pH 4.2 at four different concentrations of ABP. The points are experimental and the lines are non-linear least square fits of the data to equation 10.

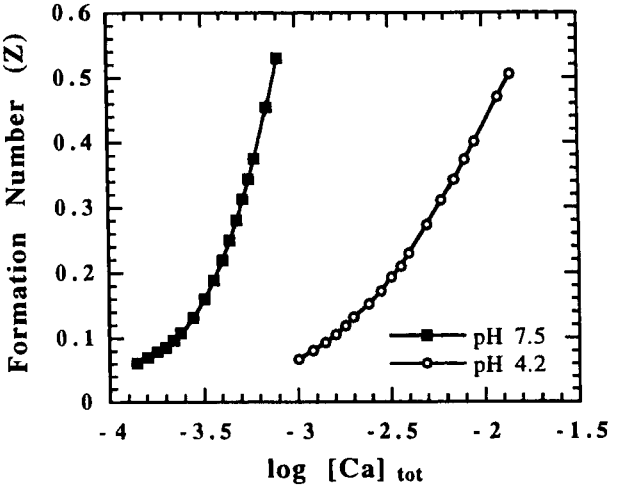

FIGURE 2

Comparison of the formation numbers, $Z = ([Ca^{2+}]_{total}-[Ca^{2+}]_{free})/[ABP]_{total}$ at pH 4.2 and 7.5 at 25°C. $[ABP]_{total} = 1 \times 10^{-3}$ M.

acceptable for SC/IM application because the acidity of the unbuffered ABP_2Ca suspensions may cause pain and local irritation. Partial neutralization of ABP_2Ca suspensions with NaOH produced unsatisfactory results for the following reasons: (i) the solubility of ABP in the partially neutralized ABP_2Ca suspensions with satisfactory pH (6-7) increased to >1.8 mg/ml, a level that causes pain in acute pain models, (ii) promotion of the dissolution of crystalline ABP_2Ca and simultaneous precipitation of amorphous material ($ABPCa_x$), and (iii) after the addition of base the time required for the pH of the suspensions to stabilize was more than three days. These results are consistent with equation 12.

$$ABP_2Ca + NaOH \longrightarrow ABP^- + ABP^{2-} + Na^+ + Ca^{2+} \quad (12a)$$

$$ABP^{n-} + x\,Ca^{2+} \longrightarrow ABPCa_x\,(\text{amorphous}) \quad (12b)$$

A possible solution to this problem was an extemporaneous suspension formulation in which ABP_2Ca would be suspended immediately prior to use in a vehicle containing a buffering agent which does not increase the solubility of ABP above 1 mg/ml and which is capable of maintaining the pH near neutrality for a prolonged period of time. Citrate and phosphate, two pharmaceutically acceptable and commonly used buffers in the neutral pH range, could not be used as buffering agents for ABP_2Ca suspensions since they compete for Ca^{2+} complexation with ABP and therefore increase the solubility of ABP.[7] Sodium acetate (NaOAc) was selected as a pharmaceutically acceptable weakly complexing buffering agent.[7] The concentration of NaOAc in the test vehicles was varied, while the total ionic strength was maintained isotonic with NaCl. The pH of these vehicles was monitored continuously during and after the addition of crystalline ABP_2Ca. The initial pH of the solutions containing NaOAc was ~7.5. Upon addition of ABP_2Ca, the pH immediately dropped to values ranging between 6.1-6.6, and continued to very slowly drift towards more acidic values over a period of several hours.

Undissolved solid was isolated from suspensions and examined by optical microscopy. An increasing proportion (20-40%) of amorphous precipitate was observed with increasing NaOAc concentration. Although the change in particle characteristics from crystalline to amorphous upon suspension of ABP_2Ca in acetate containing vehicles might seem disturbing, such changes would almost certainly take place at the physiological pH at the injection site. An isotonic vehicle containing 0.077 M NaOAc and 0.077 M NaCl, optimized by addition of 1.0% sodium carboxymethylcellulose as a

viscosity modifier was selected as a candidate for disposition, safety, and efficacy studies based on the following arguments. In this vehicle the change in particle characteristics was sufficiently slow and relatively small (<20% amorphous after 1.6 hours) to allow extemporaneous preparation of the formulation. At the same time, the buffering capacity was satisfactory (pH = 6.1-6.4) and the ABP solubility of ~0.6 mg/ml did not exceed the targeted 1 mg/ml. It was established that micronized ABP_2Ca required for this formulation can be dry heat sterilized at 160°C for 3 hours without any chemical degradation or discoloration.

Preparation of $ABPCa{\cdot}H_2O$ Salt and its Suspensions - Crystalline $ABPCa{\cdot}H_2O$ is formed according to equation 13. The experimental details are described in the materials section.

$$ABP^{2-} + Ca^{2+} + H_2O \longrightarrow ABPCa{\cdot}H_2O(s) \qquad (13)$$

The pH of $ABPCa{\cdot}H_2O$ suspended in water or saline is 6.9±0.3. The solubility of $ABPCa{\cdot}H_2O$ in water and saline is 0.1 mg/ml expressed as ABP. Since both the intrinsic pH and aqueous solubility of $ABPCa{\cdot}H_2O$ were in the range considered acceptable for a suspension formulation, a simple isotonic vehicle consisting of 0.9% NaCl and 0.5% sodium carboxymethylcellulose viscosity modifier was used. A suspension of $ABPCa{\cdot}H_2O$ can be sterilized by autoclaving at 123°C for 45 minutes without any apparent change in pH, ABP solubility or particle size.

Suspensions of $ABPCa_x$ Amorphous Salts - At pH >4 and room temperature, ABP and Ca^{2+} form finely dispersed floculant precipitates of amorphous $ABPCa_x$ salts. Since the *in-situ* formed suspensions of $ABPCa_x$ salts possessed excellent physical characteristics initially after preparation, emphasis was placed on identifying conditions under which a physically stable suspension could be prepared with satisfactory long term properties including pH and ABP solubility. Suspensions for the initial study were prepared by neutralizing ABP with base to a known degree followed by the addition of $CaCl_2$ in such proportions that the concentrations of HO^- used to neutralize ABP and of the added Ca^{2+} were equivalent, i.e. negative charge on ABP = $[HO^-] = 2[Ca^{2+}]$ (Equation 14).

$$ABP + 2x\,HO^- + x\,CaCl_2 \longrightarrow ABPCa_x + 2x\,Cl^- \qquad (14)$$

All suspensions contained 5.0 mg/ml of ABP. The pH and concentrations of ABP and Ca^{2+} in the supernatant phase as a function of the composition of the suspension are shown in Figure 3.

Examination of Figure 3 shows that suspensions with ABP/Ca ratios of 1/1.1 to 1/1.4 have pH in the desired 6-7.5 range. The solubility of ABP in the supernatant phase of those suspensions is below the targeted maximum of 1 mg/ml, and the concentrations of both total and free Ca^{2+} are ~20-50% of normal physiological plasma levels, which is considered acceptable, since it is known that IM administration of high concentrations of Ca^{2+} salts causes irritation at the injection site.[8] From the known mass balance and the concentrations of ABP and Ca^{2+} in the supernatant phase, the composition of the precipitate has been calculated. In the pH range ~5-8 the composition of all precipitates is very similar with a Ca : ABP ratio in the 1.25-1.45 range. The salts of this composition are quite basic (intrinsic pH > 9), yet they are in equilibrium with solutions having a pH as low as 5. This finding indicates that more acidic (neutral) amorphous salts can not be prepared using this method.

The main concern regarding preconstituted suspensions of amorphous $ABPCa_x$ salts was long term physical stability. After several days 4-amino-1-hydroxypropane-1,1-bisphosphonic acid calcium salt showed the tendency to form gels.[1] In our case, however, suspensions having a pH of about 7 did not form gels for periods up to two years. This was fortuitous since these suspensions have both a pH and ABP solubility in the desired range.

After the initial study, a more extensive experiment was performed. Sixty different suspensions containing 10 mg/ml of ABP were prepared in an identical way as those described above, except that in this study the negative charge on ABP was not always compensated by the addition of an equivalent of Ca^{2+}. The Ca^{2+}/HO^- and Ca^{2+}/ABP molar ratios employed to prepare the suspensions and the concentrations of ABP and the pH values in the supernatant phase of the resulting suspensions are summarized in Table 1.

Two trends were observed: (i) suspensions having a pH below ~6.5 showed the most pronounced tendency to form gels, and (ii) the solubility of ABP increased above the targeted 1 mg/ml when the negative charge on ABP was not exactly balanced by the positive charge on Ca^{2+}. The increase was dramatic when high concentrations of Ca^{2+} were used.

Based on the data shown in Table 1, suspensions with ABP : HO^- : Ca^{2+} ratios of 1 : 2.80-2.86 : 1.40-1.43 were identified as being suitable

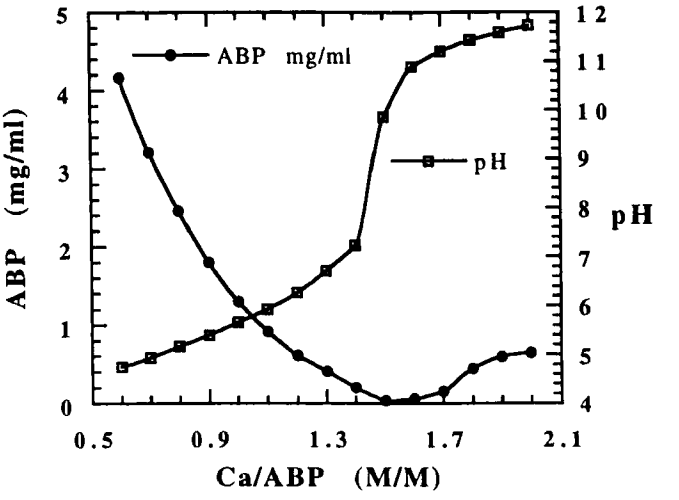

FIGURE 3
The dependence of pH and concentration of ABP in the supernatant phase of suspensions of $ABPCa_x$ amorphous salts on the $[Ca^{2+}]/[ABP]$ molar ratio used to prepare the suspensions. All suspensions contained 5 mg/ml of ABP.

for further studies. These suspensions have acceptable pH and ABP solubility, and are sufficiently removed from the region where the formation of gels was observed. The potential problem with suspensions of this composition was that they were in the region where only a small increase in the HO^-/ABP ratio can drastically increase pH (Figure 3), which could complicate reproducible preparation. A suitable buffering agent could minimize the risk of overtitrating ABP with base and shifting the pH of suspensions to the unacceptably high pH values. Citrate and phosphate buffers could not be used for the reasons previously discussed. Tris(hydroxymethyl)-aminomethane (Tris) was therefore selected as a buffer because its pK_a of 8.06 would make it largely protonated around the target suspension pH of 7.2, thus providing sufficient proton capacity to prevent shift towards higher pH values. The optimization of Tris concentration was performed by replacing a portion of NaOH used to neutralize ABP prior to precipitation with $CaCl_2$ with Tris base. Tris concentration of 1.0-1.2 mg/ml (9.1 mM) was selected as optimal since it provided sufficient buffering and acceptable pH values between 10°C and 37°C.

TABLE 1
Concentrations of ABP and pH values of supernatant phases of suspensions of $ABPCa_x$ amorphous salts prepared using varying molar ratios of $Ca^{2+}/ABP/NaOH$.

Ca^{+}/ABP (M/M)	[ABP] (mg/ml)	pH	Ca^{+}/ABP (M/M)	[ABP] (mg/ml)	pH
$Ca^{2+}/HO^{-} = 0.425$			$Ca^{2+}/HO^{-} = 0.50$		
0.77	2.73	4.97	0.90*	--	4.19
0.85	2.01	5.93	1.00*	0.86	4.87
0.94*	1.64	6.29	1.10*	0.52	5.87
1.02*	1.58	6.62	1.20*	0.41	5.98
1.11	1.53	7.18	1.30*	0.33	6.31
1.19	1.19	8.20	1.40	0.14	6.87
1.22	0.93	8.71	1.43	0.10	7.12
1.25	0.66	9.42	1.47	0.06	7.60
1.28	0.42	10.2	1.50	0.04	8.98
1.36*	0.94	11.0	1.60*	0.08	10.5
$Ca^{2+}/HO^{-} = 0.575$			$Ca^{2+}/HO^{-} = 0.65$		
1.04*	--	3.92	1.17*	--	3.76
1.15*	1.16	4.36	1.30*	1.21	4.09
1.27*	0.31	5.05	1.43*	0.35	4.64
1.38*	0.11	5.58	1.56*	0.10	5.31
1.50*	0.15	5.89	1.69*	0.62	5.72
1.61	0.43	6.41	1.82*	3.40	6.19
1.65	0.78	6.67	1.86	4.60	6.47
1.69	0.86	7.12	1.91	3.90	7.01
1.73	0.22	8.46	1.95	0.76	8.31
1.84	0.20	10.1	2.08	0.56	10.0
$Ca^{2+}/HO^{-} = 0.70$			$Ca^{2+}/HO^{-} = 0.80$		
1.31*	--	3.68	1.44*	0.95	3.61
1.45*	0.89	3.98	1.60*	1.52	3.90
1.60*	--	4.50	1.76*	--	4.38
1.74*	1.67	5.06	1.92*	--	4.89
1.89*	6.27	5.88	2.08*	2.02	6.11
2.03*	7.32	6.08	2.24	6.09	6.01
2.08	7.30	6.38	2.29	8.68	6.31
2.13	1.56	6.96	2.35	9.44	6.90
2.18	1.04	8.48	2.40	1.56	8.28
2.32	--	9.99	2.56	1.64	9.95

* these suspension formed, or showed the tendency to form gels after a period of 1 day to 4 weeks.

The suspension for long term physical testing and biological studies was prepared in the following way: calcium chloride dihydrate ($CaCl_2 \cdot 2\ H_2O$), 8.38 g, was dissolved in 200 ml of water and filtered through a 0.22 μm filter. MK-0217 (ABPNa · $3H_2O$), 13.05 g, sodium hydroxide (NaOH), 2.60 g, sodium chloride (NaCl) 2.50 g and tris(hydroxymethyl)-aminomethane (Tris), 1.00 g, were dissolved in 300 ml of water and filtered through a 0.22 μm filter. The two solutions were mixed with rapid stirring to precipitate $ABPCa_x$ amorphous salt. The volume was adjusted to almost 1 liter, and the pH was adjusted to 7.2 by dropwise addition of 1 mg/ml Tris solution. Stirring was scontinued for 1 hour to ensure that the pH of the suspension was table, the volume was made up to exactly 1 liter, and the suspension was subdivided and sterilized by autoclaving for 45 minutes at 123°C. The resulting suspension had a pH of 7.2, ABP solubility of 0.2 mg/ml, osmolarity of 290 mOsmoles, 70% of the particles were smaller than 2.5 μm, and none were larger than 5.5 μm. This preconstituted sterile suspension showed excellent physical stability under ambient conditions for at least 2 years.

BIOLOGICAL STUDIES

The formulations described above were tested in safety, disposition and efficacy studies. They all demonstrated a drastic reduction in pain on injection and tissue damaging propensity compared to the soluble salts of ABP. All three showed prolonged absorption from the injection site with the deposition of a large percentage of the dose into the bone. All three showed efficacy equal to the formulations of soluble salts of ABP. The detailed description of these studies will be a subject of separate publications.

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REFERENCES

1. Sunberg, R. J. and Benedict, J. J., European Patent Application, No. 0094714 (1983).
2. Brenner, G. S. and Ostovic, D., European Patent Application, No. 0,449,405 (1991).

3. DeMarco, J. D., Biffar, S. E., Reed, D. G. and Brooks, M. A., J. Pharm. Biomed. Analysis, 7, 1719 (1989).
4. Grabenstetter, R. J. and Cilley, W. A., J. Phys. Chem., 76, 676 (1971).
5. Wiers, B. H., J. Phys. Chem., 76 (1971) 682-687.
6. Lamson, M. L., Fox, J. L. and Higuchi, W. I., Int. J. Pharm., 21,1 43 (1984).
7. Sillén, L. G., "Stability Constants of Metal-Ion Complexes", Supplement No 1, The Chemical Society, Burlington House, London, (1971) pp. 114, 373, 836.
8. Olin, B. R. Ed., Facts and Comparisons, St. Louis, MO (updated monthly), 43.