Dissolution Tests for Self-Setting Calcium Phosphate Cement-Containing Nifedipine

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Nifedipine-containing calcium phosphate cement (CPC) was prepared, and nifedipine (NF) release from this preparation was evaluated by the shaking method (SK), Japanese Pharmacopoeia XIV (JPXIV) paddle method (PD), and JPXIV flow-through cell method (FT). The release of NF from the CPC preparation continued for 7 d or longer by all these methods. This suggests that the release of NF can be controlled by preparing NF-containing CPC. The release pattern of NF from CPC in these tests was found to follow the Higuchi equation. However, the Higuchi constant differed among the three dissolution tests, probably because the apparent tortuosity of capillary system (τ) varied.

Key words calcium phosphate cement; hydroxyapatite; dissolution test; nifedipine; flow-through cell

Self-setting type of calcium phosphate cement (CPC) was developed by Brown and Chow in 1986 on the basis of the solubility phase chart of calcium phosphate.[1] CPC can be prepared by having semi-stable type calcium phosphate transit to hydroxyapatite (HAP) by kneading it with a diluted phosphate solution. CPC preparations containing aspirin,[2] mercaptopurine,[3] cephalexin,[4] norfloxacin,[5] vancomycin,[6] insulin,[7] indomethacin,[8—10] and estradiol[11,12] have been reported. However, the methods for the dissolution tests used in these studies varied, and few systematic studies have been conducted about the release of drugs from CPC preparations. We, therefore, studied drug release from CPC preparations using nifedipine (NF) by the three dissolution test methods, *i.e.* the shaking method (SK), Japanese Pharmacopoeia XIV (JPXIV) paddle method (PD), and JPXIV flow-through cell method (FT). Presently, slow-release preparations of NF are in wide clinical use, but the development of preparations that can better control its release is still considered to be significant. In addition, NF, which is very stable to humidity,[13] was used as a model drug in this study, because this property was considered to be extremely advantageous for CPC preparations, which self-set under a high humidity condition.

Experimental

Chemicals and Materials Tetracalcium phosphate (TTCP, $Ca_4(PO_4)_2O$, Lot No. SEK 7901) for biomaterial research, dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$, Lot No. PAE 2806) and hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$, Lot No. CKE 7784) for biomaterial research were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nifedipine (NF) powder was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other solvents and reagents were commercial products of analytical grade and were used without further purification.

Preparation of CPC The calcium phosphate cement (CPC) was prepared according to the procedure described by Otsuka *et al.*[9,10] The bulk CPC powder system used was a mixture of TTCP (1.83 g), DCPD (0.86 g) and HAP 1.79 g (40%) seed crystals. The bulk cement powder (0.50 g) was mixed with 0.25 ml of 25 mM H_3PO_4 solution for 1 min to form a paste, then NF powders (1 mg (0.2%), 5 mg (1%) or 10 mg (2%)) were added to this mixture. The final paste was poured into two plastic molds (diameter 15 mm, thickness 2 mm, *i.e.* 353 mm^3/pellet), and stored in a dark desiccator at 37 °C and 100% relative humidity for 24 h. The resulting hardened CPC pellets were obtained by removing them from the mold. The weight of a CPC pellet was 250±10 mg. Transition of CPC to HAP was confirmed by powder X-ray diffraction analysis (Geigerflex RAD-C system, Rigaku Co., Tokyo, Japan) and Fourier-transformed infrared (FT-IR) spectrophotometer (FT/IR-300E, Jasco Co., Tokyo, Japan).

Dissolution Medium A simulated body fluid (SBF)[14] with ion concentrations nearly equal to those of human blood plasma was used for dissolution medium of all dissolution tests. The composition of SBF was 142.0 mM Na^+, 5.0 mM K^+, 1.5 mM Mg^{2+}, 147.8 mM Ca^{2+}, 4.2 mM HCO_3^-, 0.5 mM SO_4^{2-}, and 1.0 mM HPO_4^{2+}. NaCl, $NaHCO_3$, KCl, K_2HPO_4, $MgCl_2$, $CaCl_2$, and Na_2SO_4 were dissolved with distilled water, and this fluid was buffered at pH 7.25 with 50 mM Tris and 45 mM hydrochloric acid (HCl), and its temperature was maintained at 37 °C. The release rates from all CPC pellets containing the drug were measured as follows.

Shaking Method (SK) SK was carried out by a minor modification of the method of Otsuka *et al.*[15,16] The CPC pellet was introduced into 25 ml SBF (pH 7.25) in a 50 ml polypropylene tube with a cap. The tube was fixed on the sample holder in a thermostatically regulated water bath (Prothermo shaker NTS-211, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) maintained at 37 °C, and shaken at 90 strokes/min. During the release test, the entire dissolution medium was replaced with 25 ml of fresh buffer (SBF) at appropriate time intervals (every 3 h until after 12 h and every 12 h until after 1 week).

JPXIV Paddle Method (PD) PD was carried out by the second method using the JPXIV dissolution apparatus II (paddle method, Toyama Sangyo, Co., Ltd., Osaka, Japan). One thousand milliliters of SBF (pH 7.25) was used as a dissolution medium and the test was performed at a rotary speed of 50 rpm and 37 °C. An aliquot (1.0 ml) of the dissolution medium in the vessel was withdrawn using a pipette at appropriate time intervals (every 3 h until after 12 h and every 12 h until after 1 week) until 7 d. The same volume (1.0 ml) of fresh medium was added into the dissolution medium after each sampling.

JPXIV Flow-through Cell Method (FT) FT was carried out by the third method using the JPXIV dissolution apparatus III (flow-through cell method, Toyama Sangyo, Ltd., Osaka, Japan). The apparatus consisted of a cylindrical standard cell (22.6 mm i.d.) with a cone-shaped bottom, a constant flow pump for HPLC (BIP-I, Jasco Co., Tokyo, Japan) and a reservoir for the dissolution medium. Similar to the other methods, SBF was used as a dissolution medium, and kept at 37°C. The flow rate of the dissolution medium pumped out from the reservoir was 0.2 ml/min, because NF could not be detected by the following HPLC method at a flow rate specified by the JPXIV (4, 8, or 16 ml/min). The reduced flow rate simultaneously reduced the sample volume, which would otherwise have been very large. The dissolution medium was collected with a fraction collector (SF-2120, Advantec Co., Tokyo, Japan) after flowing through the cell every 90 min as 18-ml fractions.

Analysis of NF in Samples All drug concentrations in the samples collected by dissolution tests were determined by HPLC. The HPLC system was constructed with a Model PU-980 intelligent HPLC pump (Jasco Co., Tokyo, Japan), a Model UV-970 intelligent UV/VIS detector (Jasco Co., Tokyo, Japan), an autoinjector (SIL-6A, Shimadzu Co., Kyoto, Japan), a system controller (SCL-6A, Shimadzu Co., Kyoto, Japan) and an integrator (C-R6A Chromatopac, Shimadzu Co., Kyoto, Japan). The analytical column, Chemcosorb ODS-H (150 mm×4.6 mm i.d., particle size 5 μm, Chemco. Co., Ltd., Tokyo, Japan) was used at room temperature. The HPLC was per-

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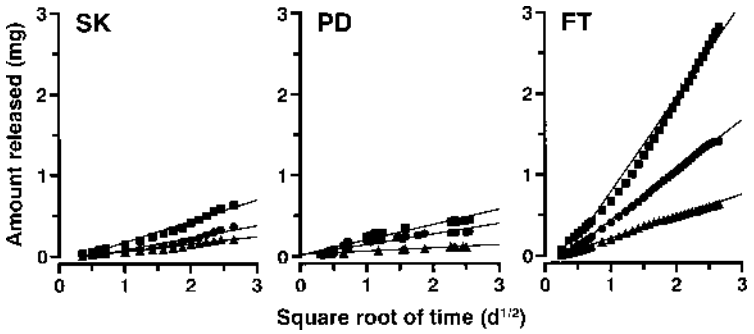

Fig. 1. Higuchi Plot for NF Release Profiles from CPC Preparations in the Three Dissolution Test Methods (SK, PD, and FT)
Symbols, amount of NF (NF contents in the matrix): ▲, 1 mg (0.2%); ●, 5 mg (1%); ■, 10 mg (2%).

Table 1. Kinetic Parameters for NF Release from CPC Preparations

NF content (mg)	Higuchi constant ($mg \cdot h^{-1/2}$)			Correlation coefficient (r^2)		
	SK	PD	FT	SK	PD	FT
1	0.0894	0.0429	0.3615	0.9806	0.9835	0.9966
5	0.2415	0.1721	0.6226	0.9668	0.9337	0.9986
10	0.3597	0.2821	1.2066	0.9856	0.9765	0.9868

formed under the following conditions by Thongnopnua *et al.*[17] The mobile phase for the assay consisted of methanol and 0.01 M ammonium acetate buffer (pH 6.1) (60 : 40, v/v) at a flow-rate of 1.0 ml/min. The column elute was monitored at wavelengths of 247 nm. A volume of 100 μl of *n*-butyl-*p*-amino benzoate solution (internal standard) in methanol at a concentration of 0.1 mg/ml was added to 1 ml of a dissolution sample into a tube and was filtered through membrane filters (0.45 μm). The aliquot of 20 μl was analyzed as the HPLC sample. For the calculation of unknown concentrations in samples, the peak-height ratios for NF relative to the internal standard were used. The peak-height ratios were linearly related (r^2=0.9996) to the amount of NF added to the dissolution fluid (SBF) in the range of 0.25 to 10 μg/ml using least square regression analysis. The coefficient of variation of the calibration curve was calculated to be less than 8%.

Results and Discussion

Three dissolution tests were performed using NF-containing CPC. The rate-limiting step for NF release from the CPC preparation was the diffusion in the micro-pores of the matrix. The drug release from the planar surface of the homogeneous drug-loaded matrix system followed the Higuchi equation[18]:

$$M(t) = \sqrt{\frac{D_i \varepsilon (2C_d - \varepsilon C_s) C_s t}{\tau}} \quad (1)$$

where $M(t)$ is the cumulative amount of drug released (until time t), D_i is the diffusion coefficient of the drug in the matrix, ε is the porosity of the matrix, C_d is the total concentration of the drug in the matrix, C_s is the solubility of the drug in the matrix and τ is the tortuosity of the capillary system.

As shown in Fig. 1, the release patterns of NF from CPC that contained NF at 1 (0.2%), 5 (1%), and 10 mg (2%) observed in the three dissolution tests were approximately linear. As shown in Table 1, a high correlation was observed between the square root of time (days) and the amount of release, and an approximately linear relationship was suggested in these tests. Therefore, the release of NF from CPC in all three tests (SK, PD, or FT) was represented by Higuchi's equation. In addition, the release of NF from CPC was confirmed to continue for 7 d or longer in all dissolution tests. These results suggest that the release of NF can be controlled by preparing NF-containing CPC. The amount of NF released was similar between SK and PD, but it was greater in FT than in the other two methods. The total volume of the dissolution medium (SBF) used during 7 d was 600 ml, 1000 ml, and 2016 ml for SK, PD, and FT, respectively. In SK, the dissolution medium was exchanged entirely at a fixed interval, and the NF concentrations in the fractions were about 3 μg/ml at the highest, which were lower than the saturation concentration of NF in SBF (10 μg/ml). In PD, the highest NF concentration in SBF was 0.49 μg/ml, which was markedly lower than the saturation concentration of NF in SBF. Therefore, the effect of the concentration of NF in the dissolution medium on the subsequent NF release was considered to be negligible. In PD, about half the volume of the dissolution medium was used compared with FT, but the ratio of the total amount of NF released between PD and FT during the 7 d was about 1/5. In SK, about 3/5 of the volume of the dissolution medium compared with FT was used, but the ratio of the total amount of NF released during the 7 d between SK and FT was about 1/4. According to these results, no clear correlation was observed between the volume of the dissolution medium and the amount of NF release so that the differences in the amount of NF released among the three test methods were not considered to be due to the differences in the volumes of the dissolution media used. The release of NF from CPC in this study is suggested to have been regulated primarily by the Higuchi equation (Eq. 1). Among the parameters in Eq. 1, only the apparent τ may be affected by the test method, and all the others are suggested to be constant. Therefore, the differences in the release pattern among these tests are suggested to have been due to differences in τ. More accurately, τ is a value specific to a given preparation and is not considered to change, but it is considered to change apparently under the influence of stirring or other maneuvers. Since the release rate of NF in the same dissolution test was suggested to be dependent on the concentration of the drug in the preparation (C_d) (Fig. 1), Eq. 2 was derived by modifying Eq. 1 to evaluate the relation between the NF contents and the Higuchi constant in CPC preparations.

$$\left[\frac{M(t)}{t^{1/2}}\right]^2 = 2\frac{D_i}{\tau}\varepsilon C_s C_d - \frac{D_i}{\tau}(\varepsilon C_s)^2 \quad (2)$$

Figure 2 shows straight lines obtained by plotting C_d along

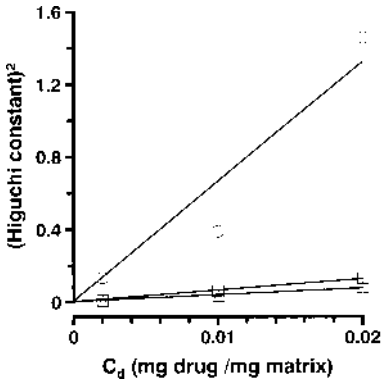

Fig. 2. Relation between the NF Contents in CPC and the Higuchi Constant

Symbols, dissolution test method (correlation coefficient): △, SK (r^2=0.9902); □, PD (r^2=0.9988); ○, FT (r^2=0.9259).

the horizontal axis and (Higuchi constant)^2 along the vertical axis using the values obtained in Fig. 1. The lines passed near the origin and showed a good linearity in all tests. This suggests that C_s is approximately zero, because C_s is markedly smaller than C_d, and that (Higuchi constant)^2 and the NF concentration in the matrix (C_d) are nearly in direct proportion. Furthermore, in the range of this experiment, various parameters involved in Higuchi's equation (τ, ε, D_i, C_s) were suggested to be constant regardless of the NF contents in the same dissolution test. In addition, the Higuchi constant was largest in FT among the three test methods. This suggests that apparent τ was smaller in FT than in the other test methods, probably because fresh dissolution medium was constantly supplied in and around the preparation.

Conclusion

Excellent slow-release preparations of NF are considered to be obtained by processing it as CPC. The dissolution pattern of CPC preparations varied among the three dissolution test methods probably due to differences in the apparent τ of Higuchi's equation. However, while the Higuchi constant differed according to the NF concentration in the matrix on the same test, the values of the parameters involved in dissolution (τ, ε, D_i, C_s) were constant under the conditions of this study. FT showed the most rapid dissolution and is considered to be advantageous for rapid evaluation of dissolution characteristics of the active drug from slowly dissolving slow-release preparations.

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