***In Vitro*–*in Vivo* Correlation of Pharmacodynamics of Felodipine in Essential Hypertensive Patients Based on an Ion-Channel Binding Model**

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The relationship between the plasma drug concentration and the antihypertensive effect of felodipine was analyzed by an ion-channel binding model which takes into consideration the slow association/dissociation process of a drug at the calcium channel. The *in vitro* dissociation constant (K_d) of felodipine to the calcium channel in the heart of rats was determined, and was compared to the *in vivo* dissociation constant ($K_{d,calc}$) estimated by the pharmacodynamic analysis of the concentration–effect data in Japanese essential hypertensive patients obtained from literature. The relative relationship between K_d and $K_{d,calc}$ of felodipine was substantially identical with eight other calcium channel blocking agents reported previously. This result suggested the possibility that we can predict the pharmacodynamic behavior of newly developed calcium channel blocking agents from the *in vitro* K_d value and plasma concentration–time profile in human using the ion-channel binding model.

Key words felodipine; calcium channel blocker; pharmacodynamic model

It is well known that several long-acting calcium channel antagonists rapidly disappear from plasma in comparison to the duration of their pharmacological effects. In our previous study, the relationship between the plasma concentration and the antihypertensive effect of eight calcium channel antagonists was analyzed by the effect compartment model and the newly developed ion-channel binding model.¹⁾ The latter includes the slow association/dissociation rate of drugs to the calcium channel, and the dissociation constants estimated from *in vivo* pharmacodynamic analysis were well correlated to the dissociation constants measured by *in vitro* binding inhibition study, suggesting that the long-acting property of these drugs is due to their high affinity to the calcium channel and slow dissociation rate. Based on the developed ion-channel binding model, we may be able to predict the pharmacological behavior of long-acting calcium antagonists from the dissociation constant measured by *in vitro* binding studies and the pharmacokinetic data obtained from clinical study. To evaluate the validity of this assumption, it is useful to apply the ion-channel binding model to other calcium channel blocking agents. In this study, we determined the inhibition constant of felodipine to the specific binding of [³H]nitrendipine to the calcium channel in the heart of rats, and compared our results to the pharmacodynamically estimated K_d value to evaluate the validity of ion-channel binding model analysis.

MATERIALS AND METHODS

Chemicals and Reagents [³H]Nitrendipine (73.5 Ci/mmol) was obtained from Amersham-Japan (Tokyo, Japan). Nifedipine, nitrendipine and felodipine were kindly provided by Bayer AG (Japan), Yoshitomi Pharmaceutical Industries, Ltd. (Japan) and Hoechst Japan, Ltd. (Japan), respectively. All other compounds were of analytical grade and were purchased from Wako Pure Chemical Industries.

Calcium Channel Blocking Activity Male Wistar rats

(300–360 g) were killed by exsanguination under ether anesthesia. The hearts were taken and homogenized in a Polytron homogenizer with 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.5) and centrifuged at $500 \times g$ for 10 min at 4 °C. The supernatant was recentrifuged at $35000 \times g$ for 10 min at 4 °C. The pellet was suspended in the initial volume of Tris–HCl buffer. This washing step was repeated twice. The final pellet was resuspended and homogenized in 25 volumes of 50 mM Tris–HCl buffer in a Teflon homogenizer. One milliliter of this homogenate was used in the binding assay.

[³H]Nitrendipine, nitrendipine, nifedipine and felodipine were dissolved in 10% methanol. To polyethylene tubes containing 50 μ l of [³H]nitrendipine (6.6 nM) and 50 μ l of the various concentrations of nitrendipine or felodipine, 1 ml of heart membrane homogenate was added. Nonspecific binding was determined in the presence of 1 μ M nifedipine. The mixture was incubated for 90 min at 25 °C. The mixture was filtered under reduced pressure in a Sampling Manifold (Millipore, Japan) through GF/C glass fiber filters (Whatman, Inc., Maidstone, England). Each filter was washed four times with 5 ml of ice-cold Tris–HCl buffer. Eight milliliters of scintillator (Scintisol EX-H, Wako Pure Chemical Industries, Ltd., Japan) were added to the filter and the radioactivity was measured by a liquid scintillation counter. The assay procedure was always carried out in duplicate.

The calcium channel blocking activity of nitrendipine was estimated by Eq. 1.

$$C_b = \left(\frac{n_{app,1} \cdot C_f}{K_{d,1} + C_f} + \frac{n_{app,2} \cdot C_f}{K_{d,2} + C_f} \right) \quad (1)$$

where $n_{app,1}$ and $K_{d,1}$ represent the number of binding sites and the dissociation constant for the high-affinity site, respectively, and $n_{app,2}$ and $K_{d,2}$ are those for the low-affinity site ($K_{d,1} < K_{d,2}$) connecting the free drug concentration (C_f) to the binding drug concentration (C_b).

The IC_{50} value of felodipine under the condition of a low concentration of nitrendipine (high-affinity site) was

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estimated by fitting Eq. 2 to the data:

$$B_{\text{felo}} = IC_{50} / (IC_{50} + C_{\text{felo}}) \quad (2)$$

where B_{felo} and C_{felo} are the binding ratio of [^3H]-nitrendipine and the concentration of felodipine used in the binding assay, respectively. The inhibitory constant (K_i) of felodipine was calculated as follows:

$$K_i = IC_{50} / (1 + [L^*] / K_{d,1}) \quad (3)$$

where $[L^*]$ is the concentration of [^3H]-nitrendipine.

Pharmacodynamic Analysis Based on the Ion-Channel Binding Model Pharmacokinetic and pharmacodynamic data after the single oral administration of felodipine (5 mg) in Japanese essential hypertensive patients (classification of WHO, stage I, stage II, and stage III, except the patients who had grave infirmity) were obtained from the literature.²⁾ The ages of the patients were from thirty-one to sixty-five, three males and eight females. The monitoring of the antihypertensive effect of felodipine started at eight a.m. The antihypertensive effect was expressed as the change in systolic blood pressure (mmHg) from the initial blood pressure before drug administration.

A pharmacodynamic model for the antihypertensive effect of calcium channel blocking agents was described in a previous study.¹⁾ A linear three-compartment model with first order absorption was selected as the model equation with the minimum Akaike's information criterion and was fitted to the plasma concentration curve of felodipine using the nonlinear least squares regression method.³⁾

$$C_p = -(A + B + C) \cdot \exp(-k_a \cdot t) + A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t) + C \cdot \exp(-\gamma \cdot t) \quad (4)$$

where C_p is the plasma concentration, and A , B , C , α , β , γ and k_a are hybrid pharmacokinetic parameters. In the ion-channel binding model, we assumed that the drug in plasma directly acted on the calcium channel at the target site with the second order association constant (k_{on}) and the first order dissociation rate constant (k_{off}). The differential equations for pharmacodynamic analysis are as follows:

$$\frac{d[RC]}{dt} = k_{\text{on}} \cdot [C_p] \cdot [R] - k_{\text{off}} \cdot [RC] \quad (5)$$

$$\frac{d[R]}{dt} = -k_{\text{on}} \cdot [C_p] \cdot [R] - k_{\text{off}} \cdot [RC] \quad (6)$$

where $[R]$ and $[RC]$ are the concentrations of the free calcium channel and the calcium channel blocked by the drug, respectively. The total calcium channel concentration $[R_t]$ is the sum of $[R]$ and $[RC]$ as follows:

$$[R] + [RC] = [R_t] \quad (7)$$

Rearranging Eq. 5 and Eq. 7 yields Eq. 8.

$$\frac{d[RC]}{dt} = k_{\text{on}} \cdot [C_p] \cdot ([R_t] - [RC]) - k_{\text{off}} \cdot [RC] \quad (8)$$

Assuming that the pharmacological effect E is proportional to $[RC]$ and that the maximum effect $E_{\text{max},R}$ is obtained when $[RC] = [R_t]$, the relationship between C_p and the pharmacological effect E (mmHg) is as follows:

$$\frac{dE}{dt} = k_{\text{on}} \cdot [C_p] \cdot (E_{\text{max},R} - E) - k_{\text{off}} \cdot E \quad (9)$$

Equations 4 and 9 were fitted to the mean time course for antihypertensive response²⁾ to estimate $E_{\text{max},R}$, k_{on} and k_{off} for the ion-channel binding model using a nonlinear least squares method.⁴⁾ Further, an apparent *in vivo* dissociation constant, $K_{d,\text{calc}}$, was calculated according to the following equation:

$$K_{d,\text{calc}} = k_{\text{off}} / k_{\text{on}} \quad (10)$$

RESULTS AND DISCUSSION

Figure 1 shows a typical Scatchard plot of nitrendipine. It was suggested that there were two binding sites of nitrendipine on the calcium channel. The dissociation constant of nitrendipine to the high-affinity site was 0.36 nM, which was similar to that determined by other investigators.⁵⁾ The inhibitive activity of felodipine to the binding of nitrendipine was determined as shown in Fig. 2. The inhibitory constant (K_i) of felodipine estimated by Eq. 3 was 0.29 nM. The activity was higher than the other calcium channel blocking agents, nitrendipine, nifedipine,⁵⁾ barnidipine,⁶⁾ nilvadipine,⁷⁾ and nicardipine.⁷⁾

Fig. 1. The Typical Scatchard Plot of Nitrendipine

The solid line is the fitted curve to the experimental data. The dissociation constant of nitrendipine to the high-affinity site was 0.36 ± 0.069 (mean $\pm$ S.E.) nM, and B_{max} was 88.1 ± 14.3 (mean $\pm$ S.E.) nmol/mg protein. The dissociation constant to the low-affinity site was 53.6 ± 24.3 (mean $\pm$ S.E.) nM, and B_{max} was 2.54 ± 0.81 (mean $\pm$ S.E.) μ mol/mg protein.

Fig. 2. The Typical Inhibitory Activity of Felodipine to the Binding of Nitrendipine

The solid line is the fitted curve to the experimental data.

Fig. 3. Antihypertensive Effect after Single Oral Administration of 5 mg Felodipine to Japanese Patients with Essential Hypertension

Each symbol represents the mean of eleven patients reported previously.¹⁾ Solid line indicates the fitted line according to an ion-channel binding model. The inset is the plasma concentration profile of felodipine¹⁾ and the fitted curve used by the linear three-compartment model.

Fig. 4. Relationship between *in Vitro* K_d Values and the *in Vivo* $K_{d,calc}$ Values Estimated by an Ion-Channel Binding Model Analysis

All data except for felodipine were analyzed in the previous study.¹⁾ ●, felodipine; ○, nifedipine; ▽, nifedipine; □, nilvadipine; ■, benidipine; △, manidipine; ▲, barnidipine; ◇, nitrendipine; ◆, efendipine.

However, the affinity of calcium channel blocking agents to the calcium channel are known to have tissue specificity and interspecies differences, therefore, comparison of the affinity of various calcium channel blocking agents by *in vitro* study may be required using the same tissue of the same animal to compare the intrinsic potency of these drugs.

Figure 3 shows the observed antihypertensive effects of felodipine²⁾ and the fitted lines based on the ion-channel binding model. The fitted curve of felodipine was coincident with the observed data. Therefore, the delays and duration in pharmacological effect of calcium antagonists could be explained by the ion-channel binding model as other calcium channel blocking agents.¹⁾ The estimated pharmacodynamic parameters, $E_{max,R}$, k_{on} , k_{off}

and $K_{d,calc}$ are 28.6 ± 5.2 mmHg, 0.156 ± 0.048 $\text{nm}^{-1} \cdot \text{h}^{-1}$, 0.160 ± 0.074 h^{-1} (optimal estimate $\pm$ S.D.) and 0.98 nM, respectively.

The relative relationship between the K_d value (K_i value estimated by Eq. 3), measured by *in vitro* study, and $K_{d,calc}$ estimated from *in vivo* pharmacodynamic analysis, was consistent with those of eight other calcium channel blocking agents obtained previously (Fig. 4). This result suggested that we can predict the pharmacodynamic behavior of calcium channel blocking agents from their pharmacokinetic properties and the dissociation rate from the calcium channel. Estimation of the K_d value is very important for the prediction of the pharmacodynamic property of long-acting calcium channel blocking agents such as benidipine, manidipine and felodipine, since their dissociation rates from the calcium channel were much slower compared with their elimination rate from plasma.

The ion-channel binding model does not consider the effect of plasma/serum protein binding. The dihydropyridine calcium channel blocking agents extensively bind to plasma proteins, and their unbound form may be directly related to their pharmacological effect. Further, it is said that the calcium channel protein is embedded within the lipid membrane bilayer, and the concentration in the lipid membrane may be more important.⁸⁻¹¹⁾ Therefore, in order to improve the predictability of a pharmacodynamic property, investigation into the unbound concentration in plasma and the partition to the lipid membrane is required in the future.

In conclusion, ion-channel binding model analysis may be applicable to most calcium channel blocking agents, of which the dissociation rate from the calcium channel are slow, and it is important to estimate the K_d value to predict the pharmacodynamic properties of these drugs. Since the relative relationship between K_d and $K_{d,calc}$ of felodipine was substantially identical with other calcium channel blocking agents, we can evaluate the pharmacodynamic behavior of felodipine from the K_d value and pharmacokinetic properties of this drug.

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