Comparative Pharmacodynamics of Eight Calcium Channel Blocking Agents in Japanese Essential Hypertensive Patients

Shuji SHIMADA, Yukiko NAKAJIMA, Koujirou YAMAMOTO,1) and Tatsuji IGA

Department of Pharmacy, University of Tokyo Hospital, Faculty of Medicine, University of Tokyo, 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo 113, Japan. Received August 7, 1995; accepted November 13, 1995

The relationships between plasma drug concentration and antihypertensive effect of eight calcium channel antagonists (nicardipine, nifedipine, nitrendipine, bendidipine, manidipine, bnrnidipine, nitrendipine and efonidipine) in Japanese essential hypertensive patients were analyzed. Based on the effect compartment model, we could explain the long duration of the pharmacological effect, and there was significant correlation (r = 0.876, p < 0.05) between estimated EC50 values and the dissociation constants (Kd) obtained from in vitro binding studies. We also developed the ion-channel binding model to understand the pharmacodynamics of long acting calcium antagonists. The model was also well fitted to antihypertensive effect data. A significant correlation between the apparent in vivo dissociation constants and in vitro Kd values was observed with a slope of 1.45 (r = 0.913), suggesting that the mechanism of long-lasting antihypertensive effect of newer developed calcium antagonists is due to their high binding affinity at ion-channel sites.

Key words: calcium antagonist; antihypertensive effect; ion-channel binding; pharmacodynamic model

The primary action of 1,4-dihydropyridine calcium antagonists is the inhibition of calcium transport through the voltage-dependent type-L calcium channels in plasma membranes following the decrease of free calcium levels in the cytosol, resulting in the relaxation of the peripheral arterial vascular tone with subsequent decrease in the systemic vascular resistance and the reduction of blood pressure.2) Calcium antagonists are used for the initial therapy of essential hypertension in a clinical situation, unless contraindications exist.3) The earlier 1,4-dihydropyridine calcium antagonists nifedipine4) and nicardipine5) require administration three times a day for antihypertensive therapy, because of the short duration of their pharmacological effect. The effectiveness of once-daily administration of several newer agents (benidipine, manidipine, bnrnidipine, nitrendipine and efonidipine) was also reported. Antihypertensive effect of these drugs is very potent, the onset is slow and they are long-lasting6) relative to their short plasma elimination half lives. Recent in vitro binding study demonstrated that these long acting calcium antagonists had a slow rate of dissociation from the calcium channel.7) In this study, we developed a classical effect compartment model and an ion-channel binding model based on the slow rates of association/dissociation of the drugs at the calcium channel to understand the pharmacodynamic aspects of long acting calcium antagonists. Relationships between plasma concentration and antihypertensive effect of these drugs obtained from clinical trials (phase II) were applied to these two models. We further attempted to make a comparison between in vivo pharmacodynamic parameters and in vitro specific binding parameters.

METHODS

Data In this study we used the pharmacokinetic and pharmacodynamic data after single oral administration of nicardipine, nifedipine, nitrendipine, bendidipine, manidipine, bnrnidipine, nitrendipine and efonidipine in Japanese essential hypertensive patients (classification of WHO,9) stage I and II) from the literature.4,6,10-14) Both plasma concentration and antihypertensive effect–time profiles were extracted from the same report for all drugs. The clinical characteristics of the patients are summarized in Table 1. Since raw data were not available from some references, the analysis was based on mean data. Control study data with placebo administration were not available for all drugs, therefore the antihypertensive effect was expressed as the change in systolic blood pressure (mmHg) from the initial blood pressure before drug administration.

Table 1. Background of Subjects

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Administration</th>
<th>Age (years)</th>
<th>Sex and no. of patients</th>
<th>Height (cm)</th>
<th>Body weight (kg)</th>
<th>Severity of HT: WHO criteria</th>
<th>B.P. in control phase (mmHg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicardipine</td>
<td>30</td>
<td>N.C.</td>
<td>N.C.</td>
<td>N.C.</td>
<td>N.C.</td>
<td>N.C.</td>
<td>I or II</td>
<td>177/106</td>
<td>5</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>10</td>
<td>AM 8:00 after meal</td>
<td>42-71</td>
<td>M3, F2</td>
<td>139-163</td>
<td>44-75</td>
<td>I or II</td>
<td>161/104</td>
<td>4</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>4</td>
<td>AM 8:00 after meal</td>
<td>28-59</td>
<td>M4, F4</td>
<td>150-166</td>
<td>43-74</td>
<td>I or II</td>
<td>164/102</td>
<td>10</td>
</tr>
<tr>
<td>Benidipine</td>
<td>8</td>
<td>AM 9:00</td>
<td>38-65</td>
<td>M4, F2</td>
<td>155-176</td>
<td>56-79</td>
<td>I or II</td>
<td>152/88</td>
<td>6</td>
</tr>
<tr>
<td>Manidipine</td>
<td>20</td>
<td>After meal</td>
<td>43-74</td>
<td>M7</td>
<td>N.C.</td>
<td>66 (mean)</td>
<td>I</td>
<td>138/84</td>
<td>12</td>
</tr>
<tr>
<td>Barnidipine</td>
<td>15</td>
<td>After meal</td>
<td>34-51</td>
<td>M9</td>
<td>158-173</td>
<td>56-79</td>
<td>N.C.</td>
<td>147/88</td>
<td>13</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>10</td>
<td>AM 8:00 after meal</td>
<td>42-66</td>
<td>M3, F2</td>
<td>146-170</td>
<td>49-80</td>
<td>I or II</td>
<td>166/103</td>
<td>11</td>
</tr>
<tr>
<td>Efonidipine</td>
<td>30</td>
<td>N.C.</td>
<td>42-82</td>
<td>M6, F2</td>
<td>N.C.</td>
<td>N.C.</td>
<td>I or II</td>
<td>150/90</td>
<td>14</td>
</tr>
</tbody>
</table>

Abbreviations: HT, hypertension; B.P., blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; N.C., not cited; M, male; F, female.

* To whom correspondence should be addressed. © 1996 Pharmaceutical Society of Japan
The circadian pattern of blood pressure in essential hypertensive patients is generally recognized, however, again blood pressure data with placebo administration was not available for all drugs. Since monitoring of the antihypertensive effect of calcium antagonists started in the morning in all studies, the relative relationship between drugs can be compared, though the effect of circadian pattern was included in each group of data. The in vitro dissociation constant ($K_d$) of the drugs to the calcium channel, which was determined by in vitro binding inhibition experiments using heart and arteries of animals, was collected from the literature (Table 2).

**Pharmacokinetic/Pharmacodynamic Analysis by the Classical Effect Compartment Model** Linear one or two compartment model with first order absorption was fitted to the plasma concentration curve of each calcium antagonist using the nonlinear least squares regression method.\(^{19}\)

\[
C_p = A_1 \exp(-k_{e1} \cdot t) - \exp(-k_e \cdot t) \\
C_p = -(A_1 + A_2) \exp(-k_{e1} \cdot t) + A_1 \exp(-k_{e1} \cdot t) + A_2 \exp(-k_{e1} \cdot t) 
\]

where $C_p$, $k_e$, and $k_{e1}$ represent the plasma concentration, the elimination rate constant and first-order absorption rate constant, respectively, and $A_1$, $A_2$, $k_{e1}$ and $k_{e2}$ are hybrid pharmacokinetic parameters. The model equation with the minimum Akaike’s information criterion ($AIC$)\(^{20}\) calculated by weighted least squares with reciprocal squared observation weighing was used as the input function for an analysis based on the effect compartment model (Eq. 3, Fig. 1A).\(^{21}\) The relationship between drug concentration in the effect compartment ($C_p$, nm) and the pharmacological effect ($E$, mmHg) was expressed by the $E_{\text{max}}$ model (Eq. 4).\(^{21}\)

\[
\frac{d(C_p \cdot V_p)}{dt} = k_{e1} \cdot C_p \cdot V_p \cdot k_{en} \cdot C_v \cdot V_v \\
E = \frac{E_{\text{max}} \cdot (C_p/K_p)}{EC_{50} + (C_p/K_p)}
\]

where $k_{e1}$, $k_{en}$, $V_v$, $V_p$, $E_{\text{max}}$, $EC_{50}$ and $K_p$ represent the rate constant connecting the central compartment to the effect compartment, the rate constant for drug removal from the effect compartment, volume of distribution in the effect compartment and the central compartment, the maximum pharmacological effect (mmHg), the 50% effective concentration and the $V_v/V_p$ ratio, respectively.

**Pharmacokinetic/Pharmacodynamic Analysis by the Ion-Channel Binding Model** A pharmacodynamic model considering association/dissociation of the drug to the ion-channel, which is similar to the receptor occupation model of non-depolarizing neuromuscular blocking agents reported by d’Hollander et al., was developed.\(^{22,23}\) Equations 1 or 2 for plasma concentration profile of each drug as the input function were also used. We assumed that the drug in plasma directly acted on the calcium channel at the target site with the second order association constant ($k_{on}$, nm$^{-1}$·h$^{-1}$) and the first order dissociation rate constant ($k_{off}$, h$^{-1}$), (Fig. 1B). The differential equations for the pharmacokinetic/pharmacodynamic analysis are as follows:

\[
\frac{d[R]}{dt} = k_{on} \cdot [C_p] \cdot [R] - k_{off} \cdot [RC] \\
\frac{d[RC]}{dt} = -k_{on} \cdot [C_p] \cdot [R] + k_{off} \cdot [RC]
\]

where [R] (nm) and [RC] (nm) are the concentration of

---

**Table 2. Characterization of Specific Radioligand Binding**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tissue preparation</th>
<th>Radioligand</th>
<th>$K_i$ value (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicardipine</td>
<td>Rat aortic</td>
<td>$[^{1}H]$Nivalipine</td>
<td>6.9 ± 2.0</td>
<td>17</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Rat heart</td>
<td>$[^{1}H]$Nifedipine</td>
<td>11 ± 0.73</td>
<td>15</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Rat aortic</td>
<td>$[^{1}H]$Nifedipine</td>
<td>1.5 ± 0.2</td>
<td>17</td>
</tr>
<tr>
<td>Benidipine</td>
<td>Rat heart</td>
<td>$[^{1}H]$Benidipine</td>
<td>0.078</td>
<td>15</td>
</tr>
<tr>
<td>Mandipine</td>
<td>Rat heart</td>
<td>$[^{1}H]$Nifedipine</td>
<td>0.24</td>
<td>8</td>
</tr>
<tr>
<td>Efonidipine</td>
<td>Porcine coronary artery</td>
<td>$[^{1}H]$[P][N]200-110</td>
<td>0.45±0.19</td>
<td>16</td>
</tr>
<tr>
<td>Efonidipine</td>
<td>Rabbit aortic</td>
<td>$[^{1}H]$Nifedipine</td>
<td>1.0 ± 0.060</td>
<td>15</td>
</tr>
<tr>
<td>Efonidipine</td>
<td>Rabbit aortic</td>
<td>$[^{1}H]$Nifedipine</td>
<td>4.48±0.14</td>
<td>18</td>
</tr>
</tbody>
</table>

The values are mean±S.E. of three to five determinations.

---

![Diagram](image)

**Fig. 1. Pharmacodynamic Models Describing the Antihypertensive Effect of 1,4-Dihydropyridine Calcium Channel-Blocking Agents**

(A) Effect compartment model. (B) Ion-channel binding model.
free calcium channel and the calcium channel blocked by the
drug, respectively. The total calcium channel content
[R] \text{ (nm)} \text{ is the sum of [R] and [RC] as follows:}

\[
[R] + [RC] = [R] + [RC]
\]

Rearranging Eqs. 5 and 7 yields Eq. 8.

\[
\frac{d[RC]}{dt} = k_{on} \left[ C_R \right] (R) - [RC] - k_{off} [RC]
\]

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

\[
\frac{dE}{dt} = k_{on} \left[ C_R \right] (E_{max,R} - E) - k_{off} E
\]

Estimation of Pharmacodynamic Parameters
Equations 4 and 9 were fitted to the mean time course for
antihypertensive response. Root mean square errors
(RMSE) normalized by the standard error of the ob-
served data were calculated as the index of fitting perfor-
ance.\(^{24}\) \( E_{max,R} \), \( E_{c50} \), and \( k_{on} \) for the effect compartment
model and \( E_{max,R} \), \( k_{on} \), and \( k_{off} \) for the ion-channel binding
model were estimated using a nonlinear least squares
method.\(^{25}\) Further, apparent the in vivo dissociation
constant, \( K_{d,calc} \) was calculated according to the following
equation:

\[
K_{d,calc} = k_{off} / k_{on}
\]

RESULTS

Plasma Concentration and Antihypertensive Effect
Plasma concentration profiles of calcium antagonists
(nicardipine, nifedipine, nilvadipine, benidipine, manidi-
pine, barnidipine, nitrendipine and efonidipine) after
single oral administration to essential hypertensive pa-
tients are summarized in Fig. 2. The plasma concentration
profiles of calcium antagonists were well fitted by one or
two compartment model with the first-order absorption
process. These fitted concentrations were used as input to
the pharmacodynamic analysis. As shown in Fig. 3, the
relationship between the plasma drug concentration and
the antihypertensive effect after single oral administration
of the calcium antagonists except for manidipine and
efonidipine showed counterclockwise hysteresis, though
there were remarkable difference between drugs in the
degree of delay of the onset of action.

Analysis by the Effect Compartment Model
Since a discrepancy between the plasma concentration and
the pharmacological effect profiles were seen in all calcium
antagonists, analysis was made based on a classical effect
compartment model (Fig. 4, Table 3). The predicted pro-

![Plasma Concentration Profiles of Calcium Antagonists after Single Oral Administration to Japanese Patients with Essential Hypertension](image)

Fig. 2. Plasma Concentration Profiles of Calcium Antagonists after Single Oral Administration to Japanese Patients with Essential Hypertension

Solid lines are the fitted lines according to Eq. 1 or 2. Except for manidipine, symbols and bars are the observed values and standard errors obtained from the literature. Each point of manidipine represents the mean ± S.D. (Table 1).\(^{6,15}\) ○, nicardipine; ●, nifedipine; □, nilvadipine; ■, benidipine; △, manidipine; ▲, barnidipine; ○, nitrendipine; ●, efonidipine.
Fig. 3. Relationship between the Plasma Concentration and the Antihypertensive Effect of Calcium Antagonists after Single Oral Administration to Japanese Patients with Essential Hypertension

Symbols are the observed values obtained from the literature (Table 1). Antihypertensive effect was expressed as the change in systolic blood pressure (mmHg) from the initial blood pressure before drug administration. ○, nicardipine; ●, nifedipine; □, nilvadipine; ■, benidipine; △, manidipine; ▲, barnidipine; ◇, nitrendipine; ●, efonidipine.

file of each drug except for nitrendipine based on this model was consistent with the observed data (RMSE normalized by the standard error of the observed data were less than 0.3). Therefore, the delays in pharmacological effect could be accounted for by the understood model. Figure 5 shows a good correlation between the in vitro $K_d$ values by in vitro binding study and the in vivo EC$_{50}$ values on a log-log scale ($r=0.876$, $p<0.05$).

Analysis by the Ion-Channel Binding Model Figure 6 shows the observed antihypertensive effects and the fitted lines based on the ion-channel binding model. The fitted curve of each drug except for nifedipine was coincident with the observed data (normalized RMSE<0.3). Therefore, the delays in pharmacological effect of calcium antagonists could also be explained by the ion-channel binding model. Table 4 indicates the estimated pharmacodynamic parameters ($E_{\text{max},R}$, $k_{\text{out}}$, $k_{\text{aff}}$ and $K_d,\text{calc}$). As shown in Fig. 7, a good correlation ($r=0.913$, $p<0.05$) was observed between the in vitro $K_d$ values and the in vivo $K_d,\text{calc}$ values.

Figure 8 shows the antihypertensive effect–time profiles after repeated dosing of each calcium antagonist; these are simulated using the pharmacokinetic/pharmacodynamic parameters obtained from the ion-channel binding model analysis (Table 4). Simulation curves were coincident with the observed data of benidipine$^{16}$ and barnidipine$^{27}$ while the predicted curves of nitrendipine and manidipine were slightly underestimated as compared with the observed data,$^{11,28}$ and that for efonidipine was overestimated.$^{29}$ Since the antihypertensive effect–time profile for other drugs after repeated oral dose were not found in the literature, we could not compare the predicted and observed profiles for these drugs. Abrupt accumulation of the pharmacological effect was not observed during repeated dosing even in long acting drugs.

DISCUSSION

The antihypertensive effects of several newly developed dihydropyridine calcium antagonists have a slow-onset and are long-lasting compared with their plasma elimination half lives, and not directly related to the plasma concentration. In this analysis mean plasma concentration data was used as the input function and mean effect data was fitted to pharmacodynamic models, since individual data in Japanese essential hypertensive patients were not available for some drugs from the literature. The counter-clockwise hysteresis between the plasma concentration and the onset of antihypertensive effect was observed (Fig. 3) in all drugs except manidipine and efonidipine. First of all, we analyzed the relationship between the plasma concentration and the pharmacological effect by the classical effect compartment model.

Effect Compartment Model Analysis As shown in Fig. 4, we obtained a good fit, except for nitrendipine, between the observed data and predicted values, and could verify
the time-delays in pharmacological effects of these drugs. For nitrindipine, plasma concentration is much lower than the estimated EC₅₀ value, therefore the estimated EC₅₀ and Eₘₐₓ may be unreliable. This indicates that the antihypertensive effect of calcium antagonists was directly related to the drug concentration at the effective site, and the relationship between drug concentration at the site and the pharmacological effect can be described by a simple Eₘₐₓ model. We also obtained a significant correlation between the in vivo EC₅₀ values calculated by this model and the Kᵣ values obtained from the in vitro binding studies (Fig. 5), indicating that the drug concentration in the effect compartment reflects the drug concentration around the ion-channel sites, and the antihypertensive effect of the dihydropyridine calcium antagonists is determined by the blockade of the calcium channel.

The Eₘₐₓ,E values were substantially constant (18–62 mmHg) relative to about a 1000-fold variation in the in vitro Kᵣ values, suggesting that these drugs may act as the full agonists of calcium channel. The classical effect compartment model was suitable to explain the long-lasting effect of dihydropyridine calcium antagonist;
Fig. 6. Antihypertensive Effect of Calcium Antagonists after Single Oral Administration to Japanese Patients with Essential Hypertension
Solid lines are the fitted lines according to a ion-channel binding model (Fig. 1B). Except for manididine, symbols and bars are the observed values and standard errors obtained from the literature. Each point of manididine represents the mean ± S.D. (Table 1).\(^{6-8,10,11}\) ○, nicardipine; ●, nifedipine; □, nilvadipine; ■, benidipine; △, manididine; ▲, barnidipine; ◇, nitrendipine; ◆, efonidipine.

Table 4. Pharmacodynamic Parameters Based on Receptor Binding Model

<table>
<thead>
<tr>
<th>Drug</th>
<th>(E_{\text{max}}) (mmHg)</th>
<th>(k_{\text{on}}) (nm (\cdot) h(^{-1}))</th>
<th>(k_{\text{off}}) (h(^{-1}))</th>
<th>(K_{\text{on},\text{act}}) (nm)</th>
<th>RMSE</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicardipine</td>
<td>48 ± 8.3</td>
<td>0.022 ± 0.013</td>
<td>0.31 ± 0.29</td>
<td>14</td>
<td>0.985</td>
<td>5.4</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>33 ± 3.4</td>
<td>0.019 ± 0.0076</td>
<td>0.47 ± 0.17</td>
<td>25</td>
<td>0.299</td>
<td>15.5</td>
</tr>
<tr>
<td>Nilvadipine</td>
<td>22 ± 7</td>
<td>0.16 ± 0.075</td>
<td>0.37 ± 0.11</td>
<td>2.3</td>
<td>0.027</td>
<td>15.4</td>
</tr>
<tr>
<td>Benidipine</td>
<td>24 ± 1.2</td>
<td>2.6 ± 0.52</td>
<td>0.012 ± 0.010</td>
<td>0.0047</td>
<td>0.073</td>
<td>-0.064</td>
</tr>
<tr>
<td>Manididine</td>
<td>17 ± 0.9</td>
<td>0.81 ± 0.17</td>
<td>0.26 ± 0.07</td>
<td>0.33</td>
<td>0.015</td>
<td>0.33</td>
</tr>
<tr>
<td>Barnidipine</td>
<td>21 ± 4.4</td>
<td>0.83 ± 0.38</td>
<td>0.62 ± 0.22</td>
<td>0.75</td>
<td>0.067</td>
<td>25.2</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>31 ± 13</td>
<td>0.13 ± 0.20</td>
<td>0.89 ± 1.11</td>
<td>7.1</td>
<td>1.213</td>
<td>27.4</td>
</tr>
<tr>
<td>Efonidipine</td>
<td>23 ± 8.7</td>
<td>4.40 ± 7.83</td>
<td>9.80 ± 19.7</td>
<td>4.37</td>
<td>0.289</td>
<td>57.1</td>
</tr>
</tbody>
</table>

The values are optimal estimate ± S.D.

however, it is difficult to explain the large discrepancy of \(k_{e0}\) values among these drugs (Table 3). Since all dihydropyridine calcium antagonists used in this analysis are highly lipophilic,\(^{30}\) have small molecular weights (340—714), and large partition coefficients to the lipid membrane (2000—20000),\(^{31}\) such slow transfer rates across the plasma membrane and variation in \(k_{e0}\) values are unlikely. In the distribution clearance model reported by Jusko, the \(k_{e0}\) values involve information on the volume of the effect compartment.\(^{32}\) If the high affinity site exists in the effective site, the \(k_{e0}\) values may be underestimated.

Ion-Channel Binding Model Analysis To explain the discrepancy between antihypertensive effect and plasma concentration profile of each drug, we developed a pharmacodynamic model based on the slow association and dissociation rates of calcium antagonists to the calcium channel (Fig. 1B). This model assumes that the anti-
hypertensive effects of calcium antagonists are directly proportional to the blockade ratio (ion-channel occupancy) of the calcium channel, and the long duration of the pharmacological effect is due to the slow dissociation rate of the drugs at the calcium channel. As shown in Fig. 6, the ion-channel binding model could explain the delays in antihypertensive effect. Further, antihypertensive effects of bendipine, manidipine, barnidipine, nitrendipine and efonidipine after repeated oral administration could be simulated by using the parameters obtained from the single administration data (Fig. 8), suggesting that the ion-channel binding model may be suitable to explain the long-lasting pharmacological effect of these drugs. This result demonstrated the lack of accumulation of pharmacological effect of long-acting calcium antagonists from single to long-term repetitive administration. Since elimination of these drugs from plasma was relatively rapid, accumulation in plasma and/or tissues may not occur in long-term treatment, reducing the risk of unpredictable adverse reactions. The long-acting property of these drugs enables once-daily treatment, and lack of accumulation of pharmacological effect avoids any toxicity.

Recently Ishii and Toyama reported the association ($k_{on}$) and dissociation rate constants ($k_{off}$) of bendipine and nitrendipine in rat cardiac membranes based on in vitro experiments. The estimated in vivo $k_{off}$ values (0.012 h$^{-1}$ in bendipine and 0.89 h$^{-1}$ in nitrendipine) obtained from the ion-channel binding model analysis were 20—30 fold smaller than in vitro $k_{off}$ values (0.372 h$^{-1}$ in bendipine and 19.1 h$^{-1}$ in nitrendipine). However, the nitrendipine/bendipine ratios of in vivo $k_{off}$ values (=74) were similar to those of the in vitro $k_{off}$ values (=51). This in vivo/in vitro difference may be due to the restriction of access of the drug to the ion-channel site under the in vivo tissue or organ condition. Since dihydropyridine calcium antagonists have high lipophilicity, the high drug concentration in the lipid membrane around the binding site leads to the underestimation of the dissociation rate. Using tissue homogenates, a rapid association/dissociation rate can be observed without such effects under the in vitro condition. There was also an excellent correlation between $K_d$ values obtained by the in vitro binding studies and the $K_d$ values (the ratio of estimated $k_{off}$ to $k_{on}$), suggesting that the pharmacological effect of calcium antagonists is mainly determined by the specific binding of these drugs to the calcium channel (Fig. 7).

In this analysis we did not take the effect of plasma/serum protein binding into consideration. It is well known that the dihydropyridine calcium antagonists extensively bind to plasma proteins (nifedipine: 96—98% as the binding fraction), nicardipine: 98—99.5%, nilvadipine: 98%, nitrendipine: 98%, efonidipine: 98.4%) and efonidipine (98.4%)). Assuming that the unbound drug in plasma is pharmacologically effective, a large difference between in vivo $K_d$ values (0.0047—25 nm) based on the concentration of the unbound drugs and in vitro $K_d$ values (0.078—11 nm) should be observed. It is known that the calcium channel protein is embedded, possibly transmembranally, within
the lipid membrane bilayer.\textsuperscript{38–41} Therefore, the lipophilicity of the drugs must be taken into consideration to estimate the drug concentration around the ion-channel, since the partitioning of the calcium antagonists into the lipid bilayer may be a prerequisite for their binding to the ion-channel. Presuming the high lipid membrane/interstitial fluid partition coefficient and a positive correlation between the lipophilicity and the plasma protein binding affinity of the drugs, the total plasma concentration may reflect the drug concentration around the specific binding site of the ion-channel in the lipid membrane. Nosaka and Ishii reported that the octanol/water partition coefficients of several dihydropyridine calcium antagonists ranged from $1000-6000$.\textsuperscript{31} Further investigation into the unbound concentration in plasma and the partition to the lipid membrane is required.

Since many calcium antagonists are used as racemates and the pharmacological potency of each enantiomer is not the same, information on the pharmacokinetic properties of each enantiomer is necessary. It may be possible to elucidate the relationship between pharmacological effect and plasma protein binding or lipid solubility of these drugs by considering the interaction of each enantiomer.

In conclusion, the antihypertensive effect of the dihydropyridine calcium antagonists was pharmacodynamically analyzed by both the classical effect compartment model and the newly developed ion-channel binding model. Both models could explain the delays in pharmacological effect of the drugs, and there was no difference in the AIC values of the nonlinear least squares regression between the two. However, considering the slow dissociation rate from the calcium channel based on the \textit{in vitro} binding study and the high lipophilicity of these drugs, the ion-channel binding model might be more suitable for analyzing the relationship between the plasma concentration profiles of drugs and the pharmacological effect.

REFERENCES AND NOTES

1) Present address: Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3–1–1, Higashi-ku, Fukuoka 812–82, Japan.


