Study of the Structure-Activity Relationships of New Dihydropyridine Derivatives

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The effects of structure around the 3- and 5-alkoxycarbonyl groups and the 2-carbamate groups of 4-(2,3-dichlorophenyl)-2-carbamoyloxymethyl dihydropyridine derivatives were investigated, concerning their vasodilating action, plasma level, hypotensive action and acute toxicity. High negative linear correlation was found between the plasma levels and the number of C atoms in esterifying alkyl groups in 3- and 5-positions of the dihydropyridine ring. High plasma levels and effective hypotensive action were found in compounds having lower alkyls as esterifying groups of 3- and 5-carboxyl groups, among 2-unsubstituted derivatives.

Keywords — calcium antagonist; structure-activity relationship; dihydropyridine; NPK-1886 (NB-818); carbamate; pharmacology

Introduction

A group of drugs called calcium antagonists (calcium entry blockers) inhibit the influx of extracellular calcium into vascular and myocardial smooth muscles through the transmembrane calcium channel, and thereby have dilating action on peripheral vessels and reduce cardiac contraction.¹⁻³⁾ Nifedipine, nicardipine, diltiazem and verapamil are examples of these drugs which are currently used for treatment of hypertension, angina pectoris, cardiac arrhythmias⁴⁾ and cerebral circulation disturbances.^{5,6)} These drugs are classified into dihydropyridine (DHP) and non-DHP groups. They differ in tissue selectivity and potency, and the former have been found to be more specific in vasodilating action.⁷⁻⁹⁾

In the course of the studies on the structure-activity relationship of DHP derivatives,^{8,10-14)} we have found several highly selective and long-lasting DHP-type calcium antagonists, and reported the calcium entry blocking and antihypertensive action^{15,16)} and the effect on the cerebral circulation¹⁷⁾ of NPK-1886 (NB-818). Here we report a structure-activity relationship centered around NPK-1886 (NB-818).

Materials and Methods

The experimental animals used were spontaneously hypertensive rats (SHR, males, 13—20 weeks old) for testing antihypertensive effects, albino rabbits (males and females, about 1.5 kg in weight) for Langendorff's heart and plasma drug level test, and male ddY mice (20 ± 2 g in weight) for the acute toxicity test.

1. Measurement of Coronary Flow — Langendorff's heart was prepared by cannulating the aorta of a rapidly isolated rabbit heart. Well-oxygenated Krebs-Henseleit solution (composition in mM: NaCl 115; KCl 4.7; CaCl_2 3.2; MgCl_2 1.2; KH_2PO_4 1.2; NaHCO_3 24.9 and glucose 10.0) at 30 ± 1 °C was perfused into the vascular bed of the heart.

The negative logarithm of the concentration of the test substances showing a 50% increase in perfusion rate (pI_{50}) was used as an index of the vasodilating potency.

2. Acute Toxicity — The lethal dose of the test compounds was calculated from experimental data on healthy mice by the up and down method.¹⁸⁾ The mice were fed solid food (Clea, CA-1) and water *ad. lib.* The animal-room temperature and humidity were controlled at 22 ± 2 °C and $55 \pm 5\%$, respectively. The drugs were

Fig. 1. Structures of the 1,4-DHP Derivatives

injected into the middle caudal vein.

3. Plasma Level — Rabbits fasted for more than 18 h except for free access to water were given 50 mg/kg of the test compounds orally using a catheter. Blood was collected in heparinized tubes from an ear vein at 0.5, 1 and 2 h after administration and centrifuged (3000 rpm, 15 min) to obtain the plasma.

The plasma levels of the test compounds were determined by high performance liquid chromatography (HPLC; Waters, μ -Bondapak C₁₈).

4. Measurement of Blood Pressure — SHR rats fasted for more than 18 h except for free access to water were warmed up in a box maintained at 37 °C for 15 min prior to the experiments.

The systolic blood pressure in the tail arteries of rats was measured using a plethysmographic system (Ueda Co. VSM-10-S) connected to a tail-cuff. The maximum pressure decrease which occurred within 6 h after oral administration was measured.

5. Drug Preparation — NPK compounds (such as, NPK-1886 (NB-818)), nifedipine, nicardipine and felodipine were synthesized in the Okazaki Research Laboratories, Banyu Pharmaceutical Co., Ltd. Test drugs dissolved in polyethylene glycol 400 (PEG 400, Wako Pure Chem. Co.) or in dimethylsulfoxide (Wako

Pure Chem. Co.) were used in the *in vivo* and *in vitro* experiments, respectively. Experiments using nifedipine *in vitro* were performed in a dark room under a sodium lamp.

Results

1. Action on Coronary Blood Flow

The perfusion rate of coronary blood flow was about 40 ml/min. By substitution of two H atoms in the 4-phenyl group of the NPK compounds (see Table I) with two Cl atoms, 2,3-dichloro, 3,4-dichloro and 2,4-dichloro derivatives were prepared, which showed the activity (pI₅₀) decreasing in this order. Among N-alkyl substituted 2-carbamate derivatives, isopropyl- and 2-propynyl derivatives (**26** and **30**, respectively: numbers indicate compound numbers in Table I) showed high pI₅₀ values among the monosubstituted derivatives, as did dimethyl- and *n*-butylmethyl derivatives (**31** and **32**) among the di-substituted derivatives. Increasing the number of C atoms in one of the esterifying alkyl groups, R₅, tended to decrease the activity, and those having Me or Et groups showed higher activities (**1**, **5–12**, **24–27**, **30–33**, **35**) than those having Pr or Bu groups (**14**, **18**, **20**).

Changing the alkyl group of the other ester group, R₃, produced no systematic effect on the activity; the activity data showed no statistically

TABLE I. Chemical Structures of R₁, R₂, R₃, R₄ and R₅ and the Biological Activities

No.	Compd. No.	R ₄	R ₅	R ₃	R ₁	R ₂	pI ₅₀	LD ₅₀ (mg/kg i.v.)	Plasma level (μg/ml)	Hypo- tensive action (ED ₂₀ ; mg/ kg <i>p.o.</i>)
1	NPK-1862	2,3-Cl ₂	-Et	-Et	-H	-H	6.98	15	1.30	20
2	NPK-1863	2,4-Cl ₂	-Et	-Et	-H	-H	5.70	50		
3	NPK-1864	2,6-Cl ₂	-Et	-Et	-H	-H	(NT)	(NT)	(NT)	(NT)
4	NPK-1865	3,4-Cl ₂	-Et	-Et	-H	-H	5.82	33		
5	NPK-1868	2,3-Cl ₂	-Me	-Me	-H	-H	6.68	132	3.95	0.9
6	NPK-1867	2,3-Cl ₂	-Me	-Et	-H	-H	7.22	36	2.45	2.4
7	NPK-1897	2,3-Cl ₂	-Me	- <i>n</i> -Pr	-H	-H	7.00	52	1.70	2.6
8	NPK-1886	2,3-Cl ₂	-Me	-iso-Pr	-H	-H	7.09	49	1.85	1.9
9	NPK-1887	2,3-Cl ₂	-Me	-iso-Bu	-H	-H	6.91	15	0.65	
10	NPK-1866	2,3-Cl ₂	-Et	-Me	-H	-H	6.61	170	3.05	49
11	NPK-1891	2,3-Cl ₂	-Et	-iso-Pr	-H	-H	6.91	15	0.62	
12	NPK-1892	2,3-Cl ₂	-Et	-iso-Bu	-H	-H	7.14	17	0.17	
13	NPK-1881	2,3-Cl ₂	- <i>n</i> -Pr	-Me	-H	-H	6.54	18	—	
14	NPK-1882	2,3-Cl ₂	-iso-Pr	-Me	-H	-H	5.94	16		
15	NPK-1888	2,3-Cl ₂	-iso-Pr	-Et	-H	-H	6.36	28	—	
16	NPK-1893	2,3-Cl ₂	-iso-Pr	-iso-Pr	-H	-H	6.45	19	0.50	
17	NPK-1895	2,3-Cl ₂	-iso-Pr	-iso-Bu	-H	-H	6.35	23	0.06	
18	NPK-1883	2,3-Cl ₂	- <i>n</i> -Bu	-Me	-H	-H	5.93	100		
19	NPK-1884	2,3-Cl ₂	-iso-Bu	-Me	-H	-H	6.14	110	—	
20	NPK-1885	2,3-Cl ₂	- <i>tert</i> -Bu	-Me	-H	-H	5.55	105		
21	NPK-1889	2,3-Cl ₂	-iso-Bu	-Et	-H	-H	6.19	71	—	
22	NPK-1894	2,3-Cl ₂	-iso-Bu	-iso-Pr	-H	-H	6.46	19	—	
23	NPK-1896	2,3-Cl ₂	-iso-Bu	-iso-Bu	-H	-H	6.50	76	0.06	
24	NPK-1869	2,3-Cl ₂	-Et	-Et	-Me	-H	6.44	63	—	
25	NPK-1870	2,3-Cl ₂	-Et	-Et	- <i>n</i> -Pr	-H	6.37	118	—	
26	NPK-1875	2,3-Cl ₂	-Et	-Et	-iso-Pr	-H	7.03	19	—	
27	NPK-1876	2,3-Cl ₂	-Et	-Et	- <i>n</i> -Bu	-H	6.29	38	—	
28	NPK-1877	2,3-Cl ₂	-Et	-Et	-iso-Bu	-H	5.43	19		
29	NPK-1878	2,3-Cl ₂	-Et	-Et	- <i>tert</i> -Bu	-H	5.29	16		
30	NPK-1880	2,3-Cl ₂	-Et	-Et	-CH ₂ C ≡CH	-H	6.64	85	—	
31	NPK-1871	2,3-Cl ₂	-Et	-Et	-Me	-Me	6.85	27	—	33
32	NPK-1879	2,3-Cl ₂	-Et	-Et	-Me	- <i>n</i> -Bu	7.26	59	—	
33	NPK-1874	2,3-Cl ₂	-Et	-Et	-Me	-CH ₂ Ph	6.78	59	—	38
34	NPK-1872	2,3-Cl ₂	-Et	-Et	- <i>n</i> -Bu	- <i>n</i> -Bu	5.96	38		
35	NPK-1873	2,3-Cl ₂	-Et	-Et	-iso-Bu	-iso-Bu	6.37	229	—	
36	Nifedipine	<i>o</i> -NO ₂	-Me	-Me			7.06	10	3.50	1.3
37	Nicardipine	<i>m</i> -NO ₂	-Me	-CH ₂ CH ₂ N (CH ₃)CH ₂ Ph			7.15	20	(NT)	2.2
38	Felodipine	2,3-Cl ₂	-Me	-Et			7.53	16	1.25	0.8
39	NPK-1961	2,3-Cl ₂	-Me	-iso-Pr			7.41	18		(NT)

(NT): not tested. —: not detected.

pI₅₀: *n* = 3–5, plasma level: *n* = 3, hypotensive action: *n* = 4.

Fig. 2. Relations between the Number of Carbon Atoms at R₃, R₅ on the DHP Ring and (A) pI₅₀ Value in Langendorff's Rabbit Heart, (B) LD₅₀ Value in Mice, (C) Max Plasma Level in Rabbits

(A) $Y = 7.08 - 0.05X$, $r = -0.306$. (B) $Y = 115.7 - 13.5X$, $r = -0.437$. (C) $Y = 5.40 - 0.90X$, $r = -0.963$.

significant linear correlation with the sum of the number of C atoms in R₃ and R₅ (Fig. 2A). Three existing DHP drugs, nifedipine, nicardipine and felodipine all showed high activities in this test.

2. Acute Toxicity (LD₅₀)

Most mice showed static or clonic convulsions and weakness after lethal doses of the test drugs. The introduction of a carbamate group in 2-position of the DHP ring tended to lower the acute toxicity, in comparison with nifedipine, nicardipine and felodipine. Also, substitution of the two H atoms in the phenyl group with two Cl atoms lowered the toxicity, in the order of 2, 4-2, 3, 4-4 and 2,3-dichloro **1** derivatives.

Among the compounds with N-unsubstituted 2-carbamate groups, those having iso-Bu, iso-Pr, or *n*-Pr groups as R₃ or R₅ group showed low toxicities. As the carbamoyl N-substituents

for R₁, iso-Pr **26**, iso-Bu **28** and *tert*-Bu **29** gave high toxicity.

When R₃ was Me and R₅ was changed, the order of LD₅₀ values was Me **5** > *n*-Pr **7** > iso-Pr **8** > Et **6** > iso-Bu **9** and, when R₅ was Et and R₃ was changed, the order was Me **10** > iso-Bu **12** > Et **1** = iso-Pr **11**.

The LD₅₀ values again showed no significant linear correlation with the sum of the number of C atoms in R₃ and R₅ (Fig. 2B). However, it was observed that the toxicity was relatively higher when the total number of C was between 4 and 6, and lower when the number of C was 2, 3 and 8.

3. Plasma Level

The maximum plasma level of each compound is shown in Table I. Plasma levels of NPK-1867, nifedipine and felodipine were

detected at 0.5 h and the other compounds were detected at 1 h. High plasma levels were observed for compounds **1**, **5–8** and **10** for which the esterifying groups R_3 and R_5 were lower alkyls. The plasma level was found to be linearly dependent on the sum of the number of C atoms (2 to 6) in R_3 and R_5 , with a high correlation coefficient $r = -0.963$ (Fig. 2C). Nifedipine and felodipine also showed high levels.

4. Hypotensive Action

The pretreatment systolic blood pressure in SHR was 197 ± 1.7 mmHg (mean $\pm$ S.E., $n = 44$). ED_{20} values, which designate the amount of drugs required for a 20% decrease in blood pressure, were 0.9 (mg/kg *p.o.*) for **5**, 2.4 for **6**, 2.6 for **7**, and 1.9 for **8**, in comparison with 1.3, 2.2 and 0.8 for nifedipine, nicardipine and felodipine, respectively. Compounds **1**, **10**, **31** and **33** also showed weak hypotensive activities.

Discussion

Past structure-activity relationship studies concerning DHP calcium antagonists^{8,10–13} have shown that (a) the 1,4-DHP ring is essential for manifestation of activity, (b) esterified carboxyl groups at the 3- and 5-positions of this ring enhance the activity, (c) an aromatic group is necessary at the 4-position of the DHP ring, and (d) when it is a phenyl group, substitution of H atom with nitro, chloro, and trifluoro groups increases the activity but substitution of nitro at the *o*-position makes the compound labile to light.

Our present study concerning the effects of structures around the 3- and 5-alkoxycarbonyl groups and the carbamate group at the 2-position of the DHP rings of 4-(2, 3-dichlorophenyl)-2-carbamoyloxymethyl-DHP derivatives gave some insight into the relationships of the structure with the *in vivo* and *in vitro* activities. These results clearly showed that the 2-carbamoyloxymethyl group decreases the toxicity of the drugs. This is illustrated by the change in the safety margin value ($[LD_{50}/\log(-pI_{50})] \times 10^7$) from 54.2 for felodipine to 59.7 for NPK-1867(**6**) and from 46.3 for NPK-1961(**39**) to 60.3 for NPK-1886(NB-818)(**8**). Since the toxicity of some calcium antagonists is related to

their hypotensive action,¹⁹ this result seems to indicate that a slow onset of hypotensive action may contribute to weak toxicity. We have earlier reported the lasting effect with slow onset of action of NPK-1886 (NB-818),^{15–17,20} and our preliminary study concerning hypotensive action and enhancement of vertebral blood flow in anesthetized dogs has shown that these actions are delayed by the introduction of 2-carbamate groups.

A definite relationship was found between the plasma level after oral administration and the groups substituted at the 2-carbamoyloxy groups. As mentioned above, a high linear correlation coefficient was found between the plasma level and the sum of the number of C atoms in the two substituted alkyl groups (Fig. 2C).

Berntsson *et al.*¹⁴ reported that the ester groups at the 3- and 5-positions of the DHP ring have significant influence on pharmacological activities in the structure-activity study of felodipine. Based on our data, this is also true for esterifying alkyl groups in 3,5-carboxy groups of 2-unsubstituted carbamate derivatives, *i.e.* the plasma level and hypotensive action are high only when the alkyl groups are Me, Et or Pr. These results form the basis for a further search for calcium antagonists with long-lasting action, since maintenance of the plasma level of drugs plays an important role like the rate of dissociation of tissue-bound drugs.²⁰

On the other hand, the number of C atoms in esterifying R_3 or R_5 groups showed no significant linear relationship with coronary vasodilating action and with acute toxicity.

We have not yet obtained a clear association between the number of C atoms and hypotensive action, because of the small number of compounds which showed this activity. However, three compounds, NPK-1867(**6**), NPK-1868(**5**) and NPK-1886(NB-818)(**8**) were found to be prominently active.

It is to be added that the 2-carbamoyloxymethyl DHP compounds that have lower alkyl groups at R_3 and R_5 are considered as potentially useful calcium entry blockers, with favorable coronary vasodilating action, plasma level maintenance, hypotensive action and low acute

toxicity. Further pharmacological studies are scheduled for these compounds.

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