Pharmacodynamic and Pharmacokinetic Studies on Prizidilol and Nipradilol (K-351), Antihypertensive Drugs with Combined Vasodilator and β-Adrenoceptor Blocking Actions, in Rabbits

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Abstract—Effects of prizidilol and nipradilol (K-351), β-adrenoceptor antagonists with vasodilator action, on blood pressure and heart rate were studied in normotensive conscious rabbits after i.v. administration. In addition, we investigated relationships between plasma drug concentrations and β-adrenoceptor blocking activity as estimated by the inhibition of isoproterenol-induced tachycardia and vasodilator activity as assessed by the inhibition of pressor response to angiotensin II (ANG II). Prizidilol (4 mg/kg) produced a significant and sustained fall in blood pressure and a slight increase in heart rate, while hydralazine (2 mg/kg) caused the same degree of hypotension and a marked tachycardia. Nipradilol (1 mg/kg) caused a significant reduction of resting heart rate, but had no significant effect on blood pressure. Propranolol (1 mg/kg) did not affect resting blood pressure and heart rate. Hypertensive response to ANG II was significantly attenuated only by hydralazine. Isoproterenol-induced tachycardia was significantly suppressed by prizidilol, nipradilol and propranolol. Good correlations were observed between β-adrenoceptor blocking activity and plasma drug concentrations. These data suggest that prizidilol has an advantage over hydralazine to induce less tachycardia, but still may cause a certain degree of increase in heart rate. Nipradilol has a more potent β-adrenoceptor blocking action than propranolol, while its vasodilator action is not obvious, at least in rabbits. Plasma concentrations of prizidilol and nipradilol are good indicators for β-adrenoceptor blocking activity, but not for vasodilator activity.

When a direct vasodilator drug such as hydralazine or minoxidil is used alone in the therapy of hypertension, the reflex activation of the sympathetic nervous system is generally induced and obtunds its antihypertensive effects. To optimize the therapy with vasodilator drugs, concomitant administration of a β-adrenoceptor blocking drug is generally required to prevent both tachycardia and the elevation of plasma renin activity. In addition, combination therapy of vasodilator drug with a β-adrenoceptor antagonist is expected to develop additive antihypertensive effects. As a result, compounds such as prizidilol and nipradilol [3,4-dihydro-8-(2-hydroxy-3-isopropoxy)-3-nitroxy-2H-1-benzopyran, K-351], which combine both vasodilator and β-adrenoceptor blocking actions in the same molecule, have been synthesized and extensively studied on their pharmacological properties (1–7). However, little is known about the relationships between plasma concentrations of these symbiotic drugs and their pharmacological activities.

This report describes the pharmacodynamic and pharmacokinetic relationships of prizidilol and nipradilol in comparison with those of hydralazine and propranolol in
conscious normotensive rabbits.

Materials and Methods

Procedures in animals: Studies were carried out in conscious male normotensive rabbits weighing 2.8±0.1 kg. The central ear artery and the ear vein were cannulated with polyethylene tubings (PE 50) under local anesthesia with lidocaine as has been previously reported by Kawashima and Ishikawa (8). The arterial cannula was connected to a pressure transducer (CP-01, Century Technology), and the blood pressure was recorded by a carrier-amplifier system (RP-5 and RTG-408, Nihon Kohden). Heart rate was measured from the lead I ECG using a cardiotachometer system (RB-J5, RB-5, RT-5 and KJR-4008, Nihon Kohden). Four mg/kg prizidilol, 1 mg/kg nipradilol, 2 mg/kg hydralazine, 1 mg/kg propranolol, or 0.1 ml/kg saline was administered through the venous catheter. Blood samples were obtained from the arterial catheter into plastic tubes at the various designated times for analysis of drug concentration. The plasma was separated by centrifugation at 3,000 r.p.m. for 10 min at 4°C and stored at −20°C until assayed.

Vasodilator activity: Percent inhibition of pressor response to angiotensin II (100 ng/kg) after the administration of the test drugs was used as a parameter for vasodilator activity, because hydralazine and other direct vasodilator drugs are reported to reduce the responses of arterial smooth muscle to various agonistic stimuli such as norepinephrine, epinephrine and angiotensin (9).

β-Adrenoceptor blocking activity: dl-Isoproterenol (0.2 μg/kg) was injected intravenously through the cannula before and at various times after the administration of test drugs. Percent inhibition of tachycardiac response to isoproterenol as compared to its value prior to the test drug administration was used to assess the β-adrenoceptor blocking activity.

Determination of plasma drug concentrations: Plasma concentration of prizidilol was measured by HPLC as reported by Larsson et al. (10). Nipradilol in plasma was determined as the heptafluoro-n-butyryl derivative by the selected ion monitoring method in gas chromatography-mass spectrometry (Yoshimura et al., in preparation). Hydralazine was determined after the method of Jack et al. (11) by gas chromatography with an electron capture detector. Propranolol was measured by radioimmunoassay (12).

Drugs and dosage: Prizidilol and nipradilol were kindly supplied by Smith, Kline & French and by Kowa, respectively. For the drugs with β-adrenoceptor blocking action, the doses which produce almost complete inhibition of isoproterenol-induced tachycardia at 30 min after the administration were chosen: prizidilol (4 mg/kg), nipradilol (1 mg/kg), and propranolol (1 mg/kg). The dose of hydralazine (2 mg/kg) which causes almost the same degree of hypotension as prizidilol was used in the study.

Data analysis: Significance of difference among the means was ascertained using Scheffé’s S test. Means and standard errors are presented in the text, table and figures.

The plasma drug concentration-time data were fitted to a two-compartment open kinetic model using the computer program TOPFIT (13). Kinetic parameters were calculated by standard procedures.

Linear least regression was used to examine the relationships between pharmacological activities and plasma drug concentrations during the steady-state decline.

Results

Effects on resting blood pressure and heart rate: Before the treatment with drugs, blood pressure and heart rate under the experimental conditions were 92.5±1.4 mmHg and 200±5 beats/min, respectively. The time courses of the changes in resting blood pressure and heart rate after the administration of drugs are shown in Figs. 1 and 2. Prizidilol (4 mg/kg) produced a prompt and sustained fall in blood pressure which was statistically significant at 0.5, 1, and 2 hr compared to the saline treated control (P<0.01 or 0.05), and it caused a slight but obvious increase in resting heart rate. The administration of nipradilol (1 mg/kg) resulted in an insignificant reduction of blood pressure which was maximum at 30 min and disappeared by 1 hr. Resting heart
rate was significantly reduced by nipradilol at 1, 2 and 3 hr (P<0.05). Hydralazine (2 mg/kg) caused a prompt and sustained reduction in resting blood pressure when compared to the control (P<0.05 at 0.5, 1 and 2 hr), and it caused a marked reflex tachycardia. Propranolol (1 mg/kg) did not produce any significant changes in both resting blood pressure and heart rate.

Effects on pressor response to angiotensin II: Angiotensin II (100 ng/kg, i.v.) increased blood pressure by 27.3±1.9 mmHg from the pretreatment level.

The time courses of the change in pressor response to angiotensin II after the administration of drugs are shown in Fig. 3. A slight reduction of the response was observed after the administration of prizidilol. Hydralazine produced a significant decrease in the pressor effect of angiotensin II at 0.5 and 1 hr after the administration (P<0.01). Nipradilol did not produce any significant change in the response, while propranolol caused a slight augmentation.

Effects on tachycardiac response to isoproterenol: The dose of 0.2 μg/kg isoproterenol, i.v., increased heart rate by 114±5 beats/min.

The time courses of the change in isoproterenol.
terenol-induced tachycardia after the administration of drugs are shown in Fig. 4. Prizidilol, nipradilol and propranolol caused significant inhibition of tachycardiac response to isoproterenol throughout the experimental period of 4 hr (P<0.01). Hydralazine caused an apparent inhibition of the response because hydralazine itself produced a significant reflex tachycardia, and isoproterenol could hardly induce any further increase in heart rate.

Kinetic parameters: Pharmacokinetic parameters are shown in Table 1. Drugs with a hydrazino moiety, hydralazine and prizidilol, had markedly smaller volume of distribution at steady state (Vdss) compared with those of nipradilol and propranolol. No other prominent differences were observed in pharmacokinetic parameters among the drugs.

Relationship between plasma drug concentrations and vasodilator activity: Correlations of plasma drug concentrations with the percent inhibition of pressor response to angiotensin II are shown in Fig. 5. Vasodilator activity of prizidilol did not correlate with the plasma concentrations. On the other hand, plasma concentrations of hydralazine weakly correlated with the vasodilator activity. Plasma concentrations of nipradilol and propranolol showed weak inverse correlations with the vasodilator activity.

Relationship between plasma drug concentrations and β-adrenoceptor blocking activity: Plasma concentrations of prizidilol, nipradilol and propranolol correlated well with the percent inhibition of isoproterenol-induced tachycardia (Fig. 6). The correlation of propranolol was stronger than those of prizidilol and nipradilol. The apparent inhibition caused by hydralazine weakly correlated with the plasma concentration.

Discussion

In the present study in normotensive rabbits, prizidilol (4 mg/kg, i.v.) caused a rapid and sustained fall in resting blood pressure and an insignificant but obvious

Table 1. Pharmacokinetic parameters in conscious rabbits after i.v. administration

<table>
<thead>
<tr>
<th>Drugs</th>
<th>(T_{1/2α}) (hr)</th>
<th>(T_{1/2β}) (hr)</th>
<th>Vdss (l/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prizidilol (5)</td>
<td>4 mg/kg</td>
<td>0.06±0.02</td>
<td>1.39±0.25</td>
</tr>
<tr>
<td>Nipradilol (5)</td>
<td>1 mg/kg</td>
<td>0.08±0.02</td>
<td>1.18±0.47</td>
</tr>
<tr>
<td>Hydralazine (6)</td>
<td>2 mg/kg</td>
<td>0.17±0.06</td>
<td>1.12±0.21</td>
</tr>
<tr>
<td>Propranolol (5)</td>
<td>1 mg/kg</td>
<td>0.11±0.02</td>
<td>1.54±0.26</td>
</tr>
</tbody>
</table>

Values given are means±S.E.M. Number of animals is shown in the parenthesis. Abbreviations used are: \(T_{1/2α}\), half-life of the α-phase; \(T_{1/2β}\), half-life of the β-phase; Vdss, volume of distribution at steady state.
tachycardia. The decreases in blood pressure and heart rate after the dose of prizidilol have been observed in spontaneously hypertensive (SHR) rats and in normotensive cats, while a reflex increase in heart rate associated with a fall in blood pressure has been found in renal hypertensive dogs (2). In both normotensive and hypertensive humans, a fall in blood pressure has been observed after i.v. infusion or oral administration of prizidilol, while variable effects have been reported on the heart rate (10, 14-17). These data suggest that prizidilol consistently produces a decrease in arterial pressure, but that effects on heart rate may vary depending on the species and the experimental conditions.

Prizidilol strongly suppressed the tachycardiac effects of isoproterenol throughout the experiment. In addition, there was a good correlation between \( \beta \)-adrenoceptor blocking activity and plasma drug concentrations. Thus, the observed increase in heart rate after prizidilol is likely mediated by the partial agonist action (2, 18), rather than by the insufficient suppression of reflex tachycardia. Although hydralazine caused a significant suppression of the pressor response to angiotensin II, prizidilol did not attenuate the response. This is consistent with the results observed in anesthetized cats (2).

Kou and Suzuki (6) and Uchida et al. (7) have demonstrated that nipradilol has combined \( \beta \)-adrenoceptor blocking and nitroglycerin-like direct vasodilator or \( \alpha \)-adrenoceptor blocking actions at relatively high concentrations in isolated canine blood vessels. Nipradilol is reported to undergo biotransformation into the desnitro derivative, which has about one-tenth the \( \beta \)-adrenoceptor blocking action of the parent drug, but no longer has vasodilator activity (7). The nitro moiety is therefore essential for

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Fig. 5. Relationships between percent inhibition of hypertensive response to angiotensin II and plasma concentrations of prizidilol, nipradilol, hydralazine or propranolol during steady-state decline in conscious normotensive rabbits. K-351 = nipradilol.
nipradilol to exert vasodilator action. In the present study, nipradilol (1 mg/kg, i.v.) produced a significant reduction in heart rate, but not in blood pressure. However, Uchida et al. (7) reported a significant and sustained fall in blood pressure and a reduction of heart rate in SHR rats after oral administration of nipradilol (3 and 5 mg/kg). One of the reasons of this discrepancy in the effect on blood pressure could be ascribed to the difference in the rate of biotransformation to the desnitro derivative between rabbits and SHR rats. Although data are not shown here, we observed rapid formation of the desnitro derivative in rabbits. An alternative possibility is that nipradilol might reduce blood pressure more effectively in hypertensive animals than in normotensives.

Marked inhibition of isoproterenol-induced tachycardia was observed in rabbits treated with nipradilol, while pressor response to angiotensin II was not attenuated. At higher plasma concentrations, nipradilol rather slightly augmented the hypertensive response to angiotensin II as did propranolol. Plasma drug concentration-time curves were biphasic and were best described by a two-compartment open kinetic model. Prizidilol and hydralazine had smaller values of Vdss than those of nipradilol and propranolol. This could be due to the lower lipophilicity of the compounds with the hydrazino moiety. The half-life values of these drugs at β-phase were almost similar in rabbits. There were good correlations between plasma drug concentrations and β-adrenoceptor blocking activity. On the other hand, only plasma hydralazine concentrations weakly correlated with vasodilator activity.

Our data show that prizidilol has an advantage over hydralazine to induce less tachycardia at the same level of blood pressure.
pressure decrease. However, it seems that prizidilol still might cause a certain degree of increase in heart rate, probably by its partial agonist action to $\beta$-adrenoceptor. Nipradilol has higher potency in $\beta$-adrenoceptor blocking activity than propranolol, but its vasodilator action is not obvious under the conditions in the present study. Plasma concentrations of prizidilol and nipradilol are good indicators for $\beta$-adrenoceptor blocking activity, but not for vasodilator activity. We recognize that the effects of these drugs we have demonstrated are in conscious normotensive rabbits and different results may be obtained in other species and under different conditions.

References


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