Comparative Effects of Selective T- and L-Type Calcium Channel Blockers in the Remnant Kidney Model

Karen A. Griffin, Maria Picken, George L. Bakris, Anil K. Bidani

Abstract—We have previously reported that the dihydropyridine L-type calcium channel blockers (CCBs) have an adverse impact on glomerulosclerosis (GS) in the remnant kidney model despite significant blood pressure (BP) reduction, because of the concurrent deleterious effects on renal autoregulation. The effects of the CCB mibefradil, which is ~10-fold more selective for T- than L-type channels, were compared with the L-type selective amlodipine. One week after 5/6 ablation, rats were left untreated or received mibefradil or amlodipine. Systolic BP was monitored by continuous radiotelemetry. At 7 weeks, proteinuria and percent GS were quantitated. Average BP was significantly and comparably reduced after mibefradil (141±3 mm Hg) and amlodipine (143±5 mm Hg) compared with untreated rats (188±5 mm Hg). Despite the reduction in BP, proteinuria and percent GS in the mibefradil- or amlodipine-treated groups were not significantly different from those in the untreated rats. Excellent correlations were observed between BP and GS in each group (r=0.74 to 0.85, P<0.02). However, the slope of the relationship between GS and BP (increase in percent GS/mm Hg increase in average BP) was made significantly steeper by both mibefradil (2.7±0.6) and amlodipine (1.9+0.6) as compared with untreated rats (0.7±0.2; P<0.01). Thus, at any given BP elevation, greater GS was seen in mibefradil- and amlodipine-treated rats as compared with untreated rats. Additional studies performed at 3 weeks after renal ablation showed that the ability to autoregulate renal blood flow, already impaired in untreated rats, was essentially abolished by both mibefradil and amlodipine, thus providing an explanation for the shift in the slope of the relationship between BP and GS. These data indicate that CCBs with selectivity for either the T- or L-type calcium channel fail to protect against GS despite significant BP reductions because of the similar adverse effects on renal autoregulation and BP transmission. (Hypertension. 2001;37:1268-1272.)

Key Words: glomerulosclerosis ■ nephrectomy ■ hypertension, experimental ■ telemetry ■ blood pressure ■ autoregulation

The rat remnant kidney (RK) model (produced by right nephrectomy and infarction of two thirds of the left kidney) has been extensively used to investigate the mechanisms responsible for the progressive nature of human renal disease.1–3 The model is characterized by hypertension and the development of proteinuria and progressive glomerulosclerosis (GS) of the initially normal remnant nephrons with time.1–3 Substantial evidence indicates that preglomerular vasodilation and impairment of the normally protective renal autoregulatory mechanisms lead to an exaggerated transmission of systemic hypertension and barotrauma to the renal microvasculature and resultant GS.4–5 We have previously shown that the dihydropyridine calcium channel blockers (CCBs) cause a further impairment of the already impaired renal autoregulation in this model and fail to provide renoprotection despite substantial BP reductions.6,7 The slope of the relationship between blood pressure (BP) and GS becomes significantly steeper in the dihydropyridine CCB-treated rats (increase in percent GS/mm Hg increase in average systolic BP) such that greater GS is observed at any given BP increase compared with untreated controls. Mibefradil, a CCB antihypertensive agent with significant antiproliferative activity and a 10-fold greater selectivity for T- compared with L-type calcium channels,8 has been recently reported to provide renoprotection in several models.9–11 The present studies compare the relative effects of mibefradil with those of the dihydropyridine CCB amlodipine on radiotelemetrically monitored systemic BP, renal autoregulation, and GS in the RK model.

Methods
Studies were conducted in male Sprague-Dawley rats (body weight 225 to 300 g) fed a standard (25%) protein diet (Purina) and synchronized to a 12:12 hours light (6:00 AM to 6:00 PM) and dark (6:00 PM to 6:00 AM) cycle. All rats received food and water ad libitum throughout the study.

Radiotelemetry
The rats were anesthetized with intraperitoneal sodium pentobarbital (45 mg/kg) and subjected to ~5/6 renal ablation (right nephrectomy and ligation of all but 1 posterior extrarenal branch of the left renal artery) and prepared for telemetric monitoring of BP (Data Sciences, International) at the time of the renal ablation surgery as previously described.12,13 Systolic blood pressure (SBP) in each animal was recorded at 10-minute intervals for ~7 weeks with each BP measurement representing the average of 50 to 60 individual BP readings during a 10-second interval (heart rate in rats is 300

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Renal Function and Proteinuria in Rats Undergoing BP Radiotelemetry for 7 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>3 days</th>
<th>Final (~7 wks)</th>
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<td></td>
<td>Body Wt, g</td>
<td>Protein, mg/kg</td>
<td>Body Wt, g</td>
</tr>
<tr>
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<td>258±7.5</td>
<td>2.9±0.5</td>
<td>439±12.0</td>
</tr>
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<td>332±8.2</td>
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<tr>
<td>Amlodipine (n=9)</td>
<td>250±5.0</td>
<td>2.6±0.5</td>
<td>354±19.2</td>
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*After baseline studies, all rats underwent ~5/6 ablation and underwent radiotelemetric BP monitoring for 7 weeks. After the first 7 days, rats were left untreated or received mibefradil in chow (0.1%) or amlodipine in drinking water (200 mg/L). Body wt indicates body weight.*

Radiotelemetry Studies

To determine if their antihypertensive effects were synergistic, additional rats (n=3 each) received either amlodipine (100 mg/L) or mibefradil (0.065% in chow) and were given the other CCB 1 week later. BP was reduced by another ~10 mm Hg, but all 6 rats died within 2 to 7 days after developing severe bradycardia.

Morphological Methods

The RKs were perfusion-fixed in situ and ~3-μm transverse sections stained with hematoxylin and eosin and periodic acid-Schiff were evaluated in a blinded fashion by standard morphological methods as previously described.5–7,12,13 At least 100 glomeruli in each animal were examined, and the severity of glomerular injury was expressed as the percentage of glomeruli exhibiting lesions of GS.

Analyses, Calculations, and Statistics

S_G, inulin, and urinary protein (quantitative sulfosalicylic-acid method) were measured as previously described.5–7,12,13 GFR was calculated using standard formulae. Autoregulatory index (AI) was calculated as follows: AI=[(RBF²—RBP)/RBF]÷[(RPP—RPP)/RPP].5–7,14 An AI of 0 indicates perfect autoregulation, whereas an AI of 1 indicates the vessels act as passive conduits for blood flow. All results are expressed as mean±SE. Statistical analysis was performed using ANOVA followed by Student-Newman-Keuls test.15 Linear regression analysis was used to calculate the slopes and intercepts of the relationship between BP and GS in each group.15 A P<0.05 was considered statistically significant.

Results

Radiotelemetry Studies

Table 1 shows that the initial body weight, S_G, and 24-hour urine protein excretion for the 3 groups were not significantly different from each other. Similarly, the S_G at 3 days was not significantly different between the groups, indicating comparable renal mass reduction in all groups. The final body and kidney weights, GFR, and RBF at 7 weeks were also not significantly different among the 3 groups.

Figure 1 illustrates the course of the weekly averages of SBP after ~5/6 renal ablation in untreated animals and the 2 groups treated with CCBs. For the first 7 days after renal ablation and before the initiation of antihypertensive therapy, the BP was similar in the 3 groups. By contrast, the average SBP during the final 6 weeks in both the mibefradil-(141±3 mm Hg) and the amlodipine-treated groups (143±5 mm Hg) was significantly lower than that of the untreated group (188±5 mm Hg) as well as in comparison to the average pretreatment SBP during the first week (P<0.001). But, these BP parameters were not different between amlodipine- and mibefradil-treated rats.

The protein excretion rate (mg/24 hours) and the percent glomeruli that exhibit GS in the RKs of these 3 groups after 7 weeks of radiotelemetry are presented in Figure 2. There was no statistically significant difference in proteinuria and GS between the mibefradil- and amlodipine-treated groups or compared with the untreated rats, although the amlodipine-treated rats tended to show greater GS than mibefradil-treated rats (6/9 amlodipine-treated rats, but only 3/11 mibefradil-treated rats exhibited ≥50% GS). Figure 3 shows the rela-
tionship between the average SBP and GS in individual animals of each group. A strong and direct relationship was observed within each group with the correlation coefficients (r) ranging between 0.74 to 0.85, P<0.01. However, the slopes of the relationship between GS and BP (percent increase in GS/mm Hg increase in average BP) were significantly different in mibefradil- and amlodipine-treated rats compared with the untreated control rats (P<0.01) but were not significantly different from each other. Although the mean GFR was not significantly different between the groups, a significant correlation was present between percent of GS and GFR in individual animals (r=0.63, P<0.01, n=32).

**Autoregulatory Studies**

Basal body weight, S Ca, and S CO at 3 days were not significantly different between the groups or as compared with the rats undergoing radiotelemetry studies (data not presented). However, body weight at 3 weeks in the amlodipine group (237±14.5 g) was significantly lower than in the untreated (307±18.7 g) or the mibefradil group (280±8.7 g; P<0.05). However, no significant differences between the groups were present for RBF and GFR (mL · min⁻¹ · kg⁻¹) measured at the ambient arterial pressure (AP); untreated (26.0±3.8 and 2.5±0.3), mibefradil- (23.8±1.8 and 2.9±0.4), and amlodipine-treated (20.6±2.1 and 1.8±0.3), respectively.

Ability to autoregulate RBF was impaired in all groups; ie, there were significant changes in RBF with each change in RPP in all groups (Figure 4). However, as shown by a comparison of the calculated autoregulatory indices, the impairment was significantly greater in both the amlodipine- and mibefradil-treated rats compared with untreated rats (<0.01). The differences in autoregulatory indices between amlodipine and mibefradil were not statistically significant.

**Discussion**

The results of the present study show that despite its differences in relative selectivity for T- versus L-type calcium channels, mibefradil-like amlodipine fails to provide renoprotection in the 5/6 renal ablation model despite substantial BP reduction. This failure likely represents a consequence of its concurrent adverse effects on renal autoregulation in this model, similar to that observed with the dihydropyridine CCBs in this and previous studies. Normal renal autoregulatory mechanisms, through proportionate vasoconstriction of the preglomerular vasculature, provide the primary protection against systemic hypertension by preventing its transmission to the renal microvasculature. These protective mechanisms are impaired in RKs and account for their increased vulnerability to hypertensive injury. Both amlodipine and mibefradil essentially abolish this residual renal autoregulatory capacity in the RK model. The further enhancement of BP transmission is expected to result in greater GS at any given BP elevation, which was in fact demonstrated in the

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**Figure 2.** Proteinuria and GS at ~7 weeks in 5/6 renal ablated rats that after the first week were left untreated or received mibefradil 0.1% in standard chow or amlodipine 200 mg/L in drinking water.

**Figure 3.** Correlation of the percentage of glomeruli with sclerosis at ~7 weeks in individual rats in the untreated and mibefradil- and amlodipine-treated groups, with their average SBP during the final 6 weeks and the average slope of the relationship between BP and percent GS. Linear regression analysis: (—–) untreated (n=11), r=0.74, slope 0.71±0.2, x-intercept 138 mm Hg, P<0.01; (—·) mibefradil (n=11), r=0.83, slope 2.79±0.6, x-intercept 128 mm Hg, P<0.002; (—–) amlodipine (n=9), r=0.77, slope 1.87±0.59, x-intercept 113 mm Hg; P<0.02; P<0.01 for the differences in slopes between mibefradil- and amlodipine-treated rats vs untreated rats.

**Figure 4.** RBF autoregulatory studies in the 3 groups of rats that had undergone ~5/6 renal ablation 3 weeks before. After day 7, the rats were either untreated or received mibefradil or amlodipine. Graded changes in RPP were produced by aortic miniclamps and resulted in significant changes in RBF with every change in RPP; *P<0.01, repeated-measures ANOVA. The bottom of the figure shows the calculated autoregulatory indices for changes in RPP between 140 to 100 mm Hg in the 3 groups. The autoregulatory indices were significantly greater in the mibefradil- and amlodipine-treated rats compared with the untreated rats, *P<0.01.
The present study by the steeper slopes of the relationship between BP and GS in mibebradil- and amlodipine-treated rats compared with untreated rats. Thus, the beneficial effects of BP reduction by these agents are likely counteracted by their simultaneous adverse effects on renal autoregulation, with a resultant inability to provide renoprotection in this model. Such interpretations are supported by the observations that antihypertensives, which are relatively neutral with respect to renal autoregulation such as triple therapy regimens (hydralazine, hydrochlorothiazide, and reperine) or agents that block the renin-angiotensin system (RAS), confer renoprotection in proportion to the achieved radiotelemetryically measured BP reductions in the 5/6 ablation model.13,17 That CCBs including mibebradil may sometimes have similar adverse effects on renal autoregulation in other models is suggested by their inconsistent ability to provide protection against proteinuria and glomerular injury.18–20

The present data, however, do not permit a definitive conclusion as to the degree to which the observed effects of mibebradil on BP and renal autoregulation, at least in the dosage used, are mediated by the blockade of T- versus L-type calcium channels. The mortality observed in rats given combined submaximal doses of amlodipine and mibebradil, similar to that observed in patients, suggests separate and additive cardiac effects.21 However, such an inference may not be equally valid for the BP and renal autoregulatory effects. Although the T-type calcium channels have been demonstrated in the renal vasculature,22,23 their independent role in renal hemodynamics and autoregulation, unlike that for L-type calcium channels,24,25 remains to be established. The observed differences between amlodipine and mibebradil during micropuncture studies are suggestive of such a role, but more direct and definitive evidence has yet to be obtained.

The present results are at seeming variance with some recent studies that have examined the relative renoprotective effects of amlodipine and mibebradil in other models of hypertensive renal injury using comparable dosages.10,11 In contrast to the present results, impressive renoprotection was achieved with mibebradil in both the DOCA+ salt model in the Wistar rat and the model of accelerated nephroclerosis in the spontaneously hypertensive rat (SHR) given N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase.10,11 However, dissimilar results were obtained with amlodipine in these 2 models. In contrast to the SHR+ L-NAME model, amlodipine was ineffective in protecting against proteinuria and GS in the DOCA+ salt model, although it did provide protection against vascular damage.10 In the present study, although a trend toward increased GS was observed in the amlodipine-treated rats, the differences were not statistically significant.

The reasons for the differences between the studies are not readily apparent but several possibilities exist. It is likely that the models differ in their susceptibility to hypertensive renal damage. Such differences may manifest themselves either as differences in the BP threshold at which renal damage develops and/or differences in the slope of the relationship between BP load and renal microvascular injury. For instance, substantial glomerular injury is observed at \( \approx 6 \) weeks in the 5/6 ablation model even when the systolic pressures do not exceed 175 mm Hg.5,12 By contrast, similar injury is only observed in the DOCA+ salt or the SHR+L-NAME model with systolic pressures that are significantly >200 mm Hg over several weeks.10,11 It is possible that such differences reflect differences in the degree to which BP is transmitted to the renal microvasculature as suggested by the higher glomerular capillary pressure (Ps) AP values in the 5/6 ablation model as compared with the DOCA+ salt and SHR+ L-NAME models. Such data have important implications for the relative renoprotective ability of antihypertensive agents in a given model. The degree of BP reduction necessary to achieve renoprotection may differ between models depending on the BP threshold for hypertensive renal damage. Thus, moderate BP reductions may be effective in the DOCA+ salt or the SHR+L-NAME models, whereas almost complete BP normalization seems to be necessary in the 5/6 ablation model.1,17 The observation that GS is prevented in RK rats being treated with dihydropyridine CCBs, if BP is further reduced to normotensive levels by the addition of another agent, is consistent with such an interpretation.28 Similarly, differences may exist between individual vascular segments (arteries and arterioles versus glomerular capillaries) and contribute to the differential effectiveness of amlodipine in protecting against vascular versus glomerular damage in the DOCA+ salt model.10 Similar differential effects of antihypertensive agents on vascular versus glomerular protection have sometimes been observed in other models.29

However, a precise estimate of the ambient BP load is critical for the validity of such distinctions and interpretations. Conventional tail-cuff BP measurements may not allow the detection of relevant differences in the chronic ambient BP load (exposure) within and between experimental groups.6,7,13,17 The fundamental lability of BP in the conscious unrestrained state, which seems to be further exaggerated in models of experimental hypertension, severely limits the conclusion on the basis of such conventional BP measurements. Even direct intra-arterial BP measurements, if only obtained for a limited period during the course, may not provide an accurate index of the history of BP exposure of the renal vasculature. Similarly, P\textsubscript{GC} measurements, while having the undoubted merit of providing a direct assessment of BP transmission to the renal microvasculature, nevertheless are limited by providing such data at a single time point and of being compromised by anesthesia-induced activation of neurohormonal systems including the RAS.31 Such activation has the documented potential for independent effects on segmental renal vascular resistances and P\textsubscript{GC}, and it may account for the often poor correlation between P\textsubscript{GC} and GS between and within models.1,12

Differential susceptibility to hypertensive renal damage may also stem from the differences in the degree to which individual BP-independent mechanisms contribute to such damage in different models. Such potential mechanisms include the degree of glomerular hypertrophy, activation of the RAS and the NO systems, and relative expression of transforming growth factor-\( \beta \) among others.1,12,33 Thus, the degree to which such pathogenetic effects can be modulated by the individual antihypertensives, including the CCBs, may represent another source for the observed differences between...
these models. For instance, the antiproliferative and/or relative renin-suppressive effects of mibebradil compared with amlodipine may account for the differences observed in the DOCA-salt\textsuperscript{9,10} model. By contrast, the result of RAS blockade in the RK model suggests little contribution of BP-independent mechanisms to GS,\textsuperscript{17} and despite the potential for differential effects of amlodipine and mibebradil on both hemodynamic and nonhemodynamic mechanisms, no differences were observed with respect to BP, renal autoregulation, and GS between the 2 agents. These data are consistent with the concept that GS in the RK model is primarily dependent on the degree to which the increased BP is transmitted to the renal microvasculature. Pharmacological interventions, such as CCBs, that enhance BP transmission (impair renal autoregulation) may not provide renoprotection proportionate to the BP reduction unless BP is lowered well into the normotensive range.

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**References**


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