Effects of the Novel Multiple-Action Agent Carvedilol on Severe Nephrosclerosis in Renal Ablated Rats

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ABSTRACT

Antihypertensive drugs have differing effects on renal hemodynamics and morphology. We analyzed whether the use of a new beta adrenoceptor antagonist and vasodilator, carvedilol (CVD), slows the progression of nephrosclerosis and whether the renoprotective effect as well as reduction in cardiac hypertrophy is dependent on the degree of blood pressure reduction. Fifty-four adult male Sprague-Dawley rats were distributed among five groups: group I served as untreated controls with 5/6 nephrectomy (Nx); group II, sham (no renal ablation or drug treatment); group III, CVD 5 (5/6 Nx and treatment with oral CVD at 5 mg/kg/day); group IV, CVD 10 (5/6 Nx and treatment with oral CVD at 10 mg/kg/day); and group V, CVD 20 (5/6 Nx and treatment with oral CVD at 20 mg/kg/day). Tail-cuff blood pressure and 24-hr urine samples were obtained before and at 3, 5 and 11 weeks of treatment with CVD. At the end of the study period, blood was taken to measure serum creatinine, plasma renin activity and CVD levels, as well as the remnant kidney and heart for morphological studies. There was a significant reduction in 24-hr UProtV in all the CVD-treated groups, and it was increasingly evident with the highest dose used. However, only rats receiving doses of 10 and 20 mg/kg/day of CVD exhibited significant decreases in blood pressure. Elevated serum creatinine levels seen in untreated controls were significantly decreased by CVD in treated rats ($P < .01$), indicating that glomerular filtration rate was improved by this drug. This was associated with a significant increase in $U_{\text{NaV}}$. Concomitant and significant ($P < .01$) decreases in plasma renin activity were observed in sham and CVD-treated rats. CVD-treated animals had considerably reduced renal damage ($P < .01$) and cardiac hypertrophy ($P < .01$) compared with untreated controls. These data indicate that CVD is effective in delaying progression of renal damage and provides beneficial effects in the remnant kidney and cardiac hypertrophy, even at nonhypotensive doses.

The adaptive renal response to reduction of renal mass in an animal model is hyperfiltration of the remaining nephron units. These changes appear as a consequence of modifications in the glomerular plasma flow rate and of an increase in the hydrostatic transcapillary pressure of the glomerular tuft (Hostetter et al., 1981). The intraglomerular hyperperfusion and hypertension are currently recognized as causes of proteinuria, GS and progressive uremia, which occur after renal mass reduction of >50% (Brenner, 1983). Several studies in the rat have demonstrated that after a critical reduction in functional renal mass, the previously normal nephrons undergo progressive injury over a few weeks to months (Brenner, 1983; Olson et al., 1987). On the basis of previous data, we know that at >10 weeks after surgical reduction of renal mass in rats, >50% of the existing glomeruli exhibited signs of GS with tubular atrophy (Bidani et al., 1987; Brenner, 1985; Olson et al., 1987). Rats undergoing renal mass reduction also developed systemic arterial hypertension that contributed, along with activation of the renin-angiotensin system, to the progressive renal damage (Bidani et al., 1987). The complex mechanisms responsible for this alteration continue to be controversial (Brenner, 1985). In the current study, we sought to establish whether the pharmacological control of hypertension using CVD, a new antihypertensive drug, could alter the course and protect against progression of renal disease in this remnant kidney model. CVD is considered a nonselective beta adrenoceptor antagonist that is free from any intrinsic sympathomimetic activity and has vasodilating properties, and it is currently marketed for the treatment of mild-to-moderate hypertension. CVD acts to reduce total peripheral resistance by blocking periph-

ABBREVIATIONS: CVD, carvedilol; DBP, diastolic blood pressure; GS, glomerulosclerosis; HPLC, high-performance liquid chromatography; MAP, mean arterial pressure; SBP, systolic blood pressure; $U_{\text{NaV}}$, urinary sodium excretion; $U_{\text{KV}}$, urinary potassium excretion; $U_V$, urinary flow; $U_{\text{ProtV}}$, urinary protein excretion; Nx, nephrectomy.
eral vascular alpha-1 adrenoceptors, thereby producing systemic arterial vasodilation and, at the same time, inhibiting reflex tachycardia through the blockade of myocardial beta adrenoceptors. Furthermore, CVD has been shown to produce significant cardioprotection in experimental animal models of acute myocardial infarction (McTavish et al., 1993; Ohlstein et al., 1993; Ruffolo et al., 1990), probably protecting vascular function by scavenging free radicals and enhancing the effects of nitric oxide (Lopez et al., 1995).

We therefore investigated the effects of CVD on serial changes in blood pressure and $U_{\text{PROV}}$ in 5/6 nephrectomized rats. We also were able to determine whether the renoprotective effect of CVD was related to the degree of blood pressure reduction and/or the dosage of the drug administered (nonhypotensive and antihypertensive doses). Histology of heart was also examined terminally in animals to document the effects of CVD on cardiac hypertrophy.

The Sprague-Dawley rat strain was selected for its genetic predisposition to develop an increase in glomerular capillary pressure, systemic high blood pressure and progressive glomerular damage (Weening et al., 1986).

Methods

Experiments were performed in male Sprague-Dawley rats. The body weight averaged 293 ± 6 g (mean ± S.E.). Rats were obtained from an established colony at the animal breeding center of our hospital research unit (fourth generation, progenitors imported from the United States), in which conditions are maintained in accordance with Spanish Royal Decree 223/1988 (BOE 18/4/1988) concerning the protection of experimental animals. The animal breeding center maintains a constant humidity and temperature-controlled air ventilation, with light/dark cycles of 12 hr.

All studies were performed in rats in accordance with the “Guide for the Care and Use of Laboratory Animals.” The right kidney was removed under light ether anesthesia. One week later, rats were anesthetized with thiopental sodium (50 mg/kg i.p. Pentothal; Abbott Labs, Chicago, IL), and two thirds of the left kidney underwent acute infarction by ligation of two or three first-order branches of the main renal artery, a surgical maneuver previously described (Anderson et al., 1985). The animal’s temperature was maintained at 37° to 38°C until recovery from anesthesia. After surgery, the animals were randomly assigned to five study groups. The untreated control group (group I) with 5/6 Nx (n = 10) did not receive any specific treatment. A group of rats (n = 8) underwent a ventral laparotomy under anesthesia as described, but only handling of the renal pedicle, without the removal of renal mass, was carried out as a sham model (group II). These two groups received tap water ad libitum and were administered 2.5 ml/kg of 0.5% methylcellulose vehicle orally. Group III (CVD 5) consisted of rats (n = 14) that received CVD (Coropres; Boehringer-Mannheim Biochemicals, Indianapolis, IN) at 5 mg/kg/day (nonhypotensive dose) in drinking water with a solution of methylcellulose at 0.5% to facilitate dissolution and absorption; one rat in this group died before the end of the first week of treatment and was not included in the data analysis. Group IV (CVD 10) consisted of rats in this group (n = 11) that received a 10 mg/kg/day dose of CVD. Group V (CVD 20) consisted of rats (n = 11) that received 20 mg/kg/day CVD as mentioned above. The drug was dissolved in drinking water, and the concentration was adjusted for the daily water intake. Every 48 hr, the respective drinking solutions for the different rat boxes were replaced. During the study period, rats ingested standard ad libitum chow (BK Universal, Barcelona, Spain) with 17% protein content and 0.25% sodium. Rats with an ablated kidney and sham operated were trained for awake blood pressure recording and to remain in individual metabolic cages.

The effect of CVD was assessed during the 11 weeks of continued treatment. At the end of the study, four rats from group I, three from group II and four from groups III, IV and V were placed on a temperature-regulated table, and a PE-50 tube were placed in the left femoral artery for continuous monitoring of systemic blood pressure. Using a pressure transducer connected to a direct recorder (Cardiostar CO-100; Experimetria MM, Ltd., Budapest, Hungary), we confirmed blood pressure values obtained with the tail-cuff method (r = .974). Rats of all groups were killed by decapitation, and blood samples were drawn rapidly and treated with EDTA to measure CVD and metabolite levels and for plasma renin activity. The blood samples were centrifuged immediately, and the plasma was frozen at −82°C until it could be assayed for plasma CVD levels and renin activity. Another blood sample was taken for measurement of serum creatinine. The heart and remnant kidney were obtained for histological study.

Twice a week during weeks 0 (basal period), 3, 5 and 11, readings were taken of body weight, diuresis with 24-hr urine collection, liquid intake and blood pressure by the tail-cuff method.

Awake blood pressure measurement. SBP, DBP and MAP were measured by the indirect tail-cuff technique under unstressed conditions. Before measurement, the rats were warmed with a heating pad for 3 min. Seven to 10 readings for each rat, with 2- to 3-min intervals, were taken on weeks 0, 3, 5 and 11 with a validated pressure monitor (LE-5001; Letica Scientific Instruments, Barcelona, Spain) calibrated with a mercury manometer. The mean value of the last five measurements was used as the blood pressure. The possibility of estimating MAP rather than SBP in awake rats is highly desirable because MAP is the actual driving force in the arterial circulation. The validity of the use of the tail-cuff method of blood pressure determination in renal ablated rats was determined by performing a parallel experiment cited above.

Analytical procedures. Serum creatinine was measured with a clinical analyzer (Hitachi 717; Boehringer-Mannheim Biochemica, Mannheim, Germany) by an enzymatic colorimetric test (Boehringer Mannheim). In the 24-hr urine samples, sodium and potassium were measured (mmol/liter) by selective electrode (Hitachi 717) and 24-hr urine protein concentration (mg/day) through precipitation with sulfoacetic acid at 3%. Plasma renin activity was determined using GammaCoat [125I]Plasma Renin Activity Radioimmunooassay Kit (INCSA, Stillwater, MN). Data are expressed as ng of angiotensin I/ml/hr maintained in this form. Plasma from each rat was processed to determine CVD levels in its free form and as the metabolite desmethylcarvedilol (ng/ml) by HPLC as previously described in detail (Reiff, 1987).

Morphological studies. On the day of sacrifice, animals were killed by decapitation, and the heart was removed en bloc, as well as remnant kidney; both were washed thoroughly with normal saline and fixed in 10% phosphate-buffered formalin for processing at the University of Las Palmas de Gran Canaria Histopathology Department by a pathologist without prior knowledge of the different rat groups. Kidney weight was measured after being washed with normal saline. The frequency of focal and segmental glomerular sclerotic lesions was determined by examining all glomerular profiles (average ± S.D., 82.2 ± 6.8/animal) contained in a section from each kidney. Sections were cut at thicknesses of 3 to 5 μm and stained with hematoxylin and eosin, Masson trichrome, silver methenamine and reticulin. For each animal, the number ofglomeruli with segmental lesions was expressed as a percentage of the total number of counted glomeruli. Glomerular collapse and glomerular necrosis were evaluated quantitatively in each kidney section under light microscopy. The fraction of renal cortex occupied by interstitial tissue was evaluated in Masson-stained sections.

The heart was also studied after it was weighed. The optimal views of the left ventricle were obtained by a transverse section 2 mm below the ventriculocaudicurial division. By analyzing these images with a computer-assisted unit (Image-Pro; Media Cybernetics, Inc., Silver Spring, MD), muscular thickness of the left ventricle (mm) and...
area of the left ventricular mass (mm²) were measured. Hematoxylin and eosin stain was used to study the distribution and characteristics of the cardiac fibrilla, nuclei, arterial and venous vessels and cellular infiltration.

Data analysis. The values presented are mean ± S.E. Statistical differences among the groups were evaluated by one-way analysis of variance. In addition, Student’s t test was used for paired and non-paired data with Bonferroni’s correction (Wallenstein et al., 1980) when it was necessary to evaluate the intragroup statistical significance throughout the experimental period. Statistical significance was considered at P ≤ .05.

Results

No differences in food and water intake (table 1) were detected between treated and untreated rats. The pattern of water intake was similar in each group from week 3. Weight gain over the remainder of the study showed only minor differences among the groups.

Blood Pressure, Diuresis, Natriuresis and Kaliuresis

All groups except the sham group were matched for blood pressure after renal ablation. Results of MAP measurements in awake rats are depicted in figure 1. Untreated rats exhibited sustained hypertension throughout the experiment period. Group III rats treated with 5 mg/kg/day doses of CVD did not present significant reductions in the pressure values throughout the study; in contrast, groups IV and V reduced MAP significantly by 11 weeks of treatment (P < .01, t = 6.2; P < .001, t = 7.1, respectively), although CVD antihypertensive effect became evident in those latter groups from the fifth weeks of administration (123 ± 4.3; P < .001; 113.6 ± 1.4; P < .01, respectively). Groups I, III, IV and V were also matched for UV, UNaV and UKV. Urine output and UKV were not statistically different between treated and untreated groups, whereas major differences were observed in UNaV at 11 weeks of CVD treatment (table 1). Only treated animals increased the UNaV in relation to pre-CVD values: group III (P < .001, t = −5), group IV (P < .05, t = −3.2), and group V (P < .05, t = −3).

Renal Function and Plasma Renin Activity

As expected, renal ablation increased serum creatinine in the untreated control group and unaffected by the vehicle treatment. At 11 weeks, serum creatinine concentrations in CVD-treated rats were approximately half the levels observed in the vehicle-treated animals (fig. 2a). There were no differences in the above values among those CVD-treated animals. Plasma renin activity in CVD-treated rats was not significantly different (fig. 2b). However, plasma renin activity was ~4- to ~5-fold higher in untreated controls.

| Table 1: Water intake and renal excretory function during a 24-hr period 11 weeks after treatment with CVD in rats with 5/6 nephrectomy |
|---|---|---|---|
| Group | Water intake | UV | UNaV | UKV |
| I: Untreated controls (5/6 Nx) | 41.7 ± 1.6 | 40.7 ± 7.3 | 1.63 ± 0.2 | 2.9 ± 0.16 |
| II: Sham | 47.3 ± 1.3 | 39.2 ± 1.4 | 2.42 ± 0.09 | 2.8 ± 0.17 |
| III: CVD 5 | 38.8 ± 4.9 | 26.2 ± 3.6 | 2.34 ± 0.22 | 3.0 ± 0.29 |
| IV: CVD 10 | 31.9 ± 2.1 | 27.9 ± 2.6 | 2.32 ± 0.19 | 2.9 ± 0.08 |
| V: CVD 20 | 32.1 ± 2.7 | 32.1 ± 4.7 | 2.32 ± 0.19 | 2.9 ± 0.08 |

Values represent mean ± S.E.

* P < .01, ** P < .05 different from untreated control group.
Groups I and III presented intratubular casts of protein material and cell infiltration of the interstitial area, frequently in association with tubular atrophy and vacuolization. These findings were less pronounced in groups IV and V. The extent of interstitial expansion tended to follow that of glomerular injury; the fraction of cortical parenchyma occupied by interstitial tissue was 8.3 ± 0.9% in group I and decreased to 2.1 ± 0.3% in group V (P < .05). As expected, these studies show the prevalence of segmental glomerular lesions closely linked to systemic blood pressure values and the degree of protein excretion (fig. 3). Group I rats exhibited segmental lesions in 47.5 ± 2.4% of glomeruli, associated with a higher frequency of collapsed glomeruli (1.4 ± 0.6). The prevalence of segmental glomerular lesions diminished in the groups treated with CVD (table 3); thus, in group V, rats presented 6.5 ± 1.43%, a value not statistically different from that observed in the sham group, indicating that CVD at 20 mg/kg/day provided a marked protection against glomerular structural injury (fig. 4). Glomerular collapse was largely prevented by CVD administration (group V, 0.2 ± 0.1; P < .05 vs. group I).

All animals in which renal ablation was carried out, untreated and treated with CVD had left ventricular hypertrophy when expressed as the increase of cardiac weight normalized to body weight (data not shown) compared with the sham group. Left ventricular hypertrophy, expressed as interventricular septal thickness, was evident in untreated animals (5.5 ± 0.2 mm). Morphological study provided a significant decrease in the thickness of the left ventricle wall in all CVD-treated groups, although it was more evident in group V rats (2.8 ± 0.1 mm, P < .01), with values close to those obtained in the sham group (2.5 ± 0.1 mm), as shown in figure 5. Similar results were obtained when we measured the area of the left ventricular mass, the major reduction was obtained in group V rats (116.4 ± 6.3 mm²) vs. group I rats (173.7 ± 4.1 mm², P < .01) but elevated in contrast with sham values (65.2 ± 2.9 mm², P < .01). In addition, in untreated control rats, we found myocytes increased in size and clustered with enhanced collagen accumulation, clear evidence of hypertrophy as well as a notable perivascular fibrosis in comparison with the sham group. Histological data from animals receiving the highest dose of CVD exhibited only small areas of hypertrophy with alternate zones of striking normality (images not shown).

**Discussion**

The rationale for this study is based on data showing that patients who had lost part of their functional renal mass to initial damage would inevitably progress to renal failure and that a similar course associated with development of focal glomerular sclerosis-like lesions had been observed in laboratory animals with or without surgical intervention (Anderson, 1985; Brenner, 1983; Hostetter et al., 1981; Meyer et al., 1987). As in humans, there is a varying degree of susceptibility, according to gender and strain of rats (Elema and Arends, 1975; Linkswiller et al., 1952; Weening et al., 1986), to development of segmental and focal glomerular sclerosis. Exhibition of GS in this model is preceded by systemic and glomerular hypertension in the remnant nephrons (Anderson et al., 1985) and accompanied by increasing proteinuria and progressive renal insufficiency.
The goal of this study was to more precisely define the mechanism by which CVD protects remnant glomeruli in this experimental model (Brooks et al., 1993; Rodriguez-Perez et al., 1996) and whether this was mediated by, or is independent of, the blood pressure-lowering effect. Our results indicate that oral administration of CVD prevents the rise in blood pressure, reduces the U_{prot} and provides a reduction in glomerular sclerosis, in some cases even without normalization of systemic hypertension. Furthermore, we observed that CVD is efficacious in improving left ventricular hypertrophy.

Some of the renal effects of CVD have been studied in rats and dogs (Barone et al., 1996; Brooks et al., 1993; Kohno et al., 1988; Ruffolo et al., 1990; Tamaki et al., 1988). The intrarenal and oral administration of CVD in anesthetized and conscious spontaneously hypertensive rats produced either an increase or no modification in renal blood flow despite a dose-dependent reduction in MAP, indicating that renal autoregulatory integrity was unaffected by CVD. In addition, treatment of systemic hypertension. Furthermore, we observed that CVD is efficacious in improving left ventricular hypertrophy.

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sion pressure in addition to an antinatriuretic response. Beta adrenergic stimulation, perhaps by direct beta receptor activation, might inhibit sodium reabsorption in the proximal nephron (Blendis et al., 1972); it could be predicted that beta blockade would increase proximal sodium reabsorption and thereby decrease sodium delivery to the distal tubule (Sealey and Laragh, 1974). In the remnant kidney model used in the present study, the functional comparisons between untreated and treated animals after 11 weeks of CVD were remarkably significant. Rats receiving CVD had a significantly better renal function than untreated controls, as demonstrated by a lower serum creatinine concentration. Although the levels of serum creatinine were lowest in the group treated with the highest doses of CVD, the differences were not significant compared with the other treated animals. This may indicate the renoprotective effect of CVD independent of blood pressure values. Along with this decrease in serum creatinine levels, all CVD-treated animals presented increased natriuretic activity. Despite the fact that sodium intake was not strictly monitored, we may attribute this natriuresis to a compensatory renal mechanism due to an improvement of glomerular filtration rate produced by CVD. Such an improvement in the present study is independent of blood pressure levels. A direct effect of CVD on renal tubules inhibiting tubular sodium reabsorption remains speculative, and further elucidation is required. CVD also reduced plasma renin activity in all treated rats in comparison with the untreated controls, suggesting that renoprotection and cardiac hypertrophy decrease produced by CVD could be due to the inhibition of the renin-angiotensin system. This supports the relevance of this system in the pathogenesis of renal damage and cardiac hypertrophy (Laragh et al., 1972). This mechanism may have been shared with the pure beta adrenoceptor antagonists. The protective effect of CVD does not seem to be exclusive to inhibition of the renin-angiotensin system, however, if we consider what took place when nonhypotensive doses of CVD were administered.

The beneficial effects of CVD are more similar to those produced by the angiotensin-converting enzyme inhibitors regarding proteinuria reduction and prevention of renal damage (Anderson, 1987; Hall et al., 1985) than to beta adrenoceptor antagonists, which have scarce antiproteinuric effect (Rosenberg and Hostetter, 1991) and provide less protection against renal injury (Barone et al., 1996). This difference is also evident in reference to alpha-1 adrenoceptor antagonists with sodium retention tendencies, which can achieve an equivalent antiproteinuric effect only with significant blood pressure decreases (Rosenberg and Hostetter, 1991).

Renal protective effects have also been demonstrated previously for CVD in two models of renal failure, one from Nakamoto et al. (1988), in which glomerular morphology was...
not assessed, and one from Brooks et al. (1993). Clearly, UProtV and serum creatinine were significantly reduced; however, the authors could not conclude whether the beneficial effects of CVD were related to the reduction in the arterial pressure values.

In the present study, CVD produced clear renoprotective effects, demonstrated by a lower percentage of segmental glomerular lesions associated with a decrease of collapsed glomeruli and a reduction in UProtV, even with the nonhypotensive doses. Global collapse of the tuft consistent with glomerular ischemia also appeared modestly in renal ablated rats. Because of large morphological differences between collapsed and sclerotic glomeruli, the underlying mechanisms of these two pictures appear to differ. Although the former is related to hypoperfusion of the afferent arteriole, the latter is related to high glomerular capillary pressure (Fujihara et al., 1994). Because we did not measure glomerular volume and pressure in this study, and given the complex mechanisms involved in the development and progression of chronic renal failure, the contribution of the calcium channel blockade properties (Ruffolo et al., 1990) of CVD to attenuate the global collapse of the glomeruli will have to be investigated. These actions on proteinuria and renal morphology could suggest that CVD may have specific tissue effects that are not related to arterial blood pressure reduction. Although the mechanism by which renoprotection with CVD is achieved remains to be elucidated, some of its properties, such as antiproliferation (Ohlstein et al., 1993), as a calcium channel blocker (Ruffolo et al., 1990), and as an antioxidant (Yue et al., 1992, 1995) have been discussed. An interaction of the
renin-angiotensin system with growth factors such as endothelin and the involvement of endothelin with renal disease progression have been documented (Bakris and Re, 1993; Floege et al., 1992; Orisio et al., 1993). As a premature speculation, CVD could contribute to renal protection through a blockade of the effects of endothelin-reducing intracellular Ca²⁺ movements.

Finally, as reported by Sen et al. (1974), Tamanek (1979) and Feld et al. (1981), an elevated blood pressure is not the only factor contributing to cardiac hypertrophy in the rat model. In the present study, CVD produced a marked reduction in ventricular wall thickness in relation to the control group, independent of the dosage of CVD used and blood pressure values obtained. This cannot be explained solely via hemodynamic mechanisms but might suggest that the some or all of the multiple actions of CVD are contributing to slowing the process of muscle hypertrophy. In summary, CVD, a novel multiple-action agent that is currently used for the treatment of angina and congestive heart failure (Packer et al., 1995), provides, even at nonhypotensive doses, renal protective effects. It is tempting to speculate that these beneficial effects in chronic renal failure and cardiac hypertrophy are the results of a balanced interaction between its different pharmacological properties. In addition, inhibition of the renin-angiotensin system in this model could suggest that CVD acts by blocking growth factors such as endothelin.

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