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Effect of telmisartan on preexistent cardiac and renal lesions in spontaneously hypertensive mature rats

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Summary. Fifteen adult male spontaneously hypertensive rats (one year old) (SHR) were separated into three groups (n=5 each) during 15 weeks as follows: initial control group (IC); final control group (FC); and telmisartan group (T) (1.2 mg/kg/day of telmisartan). Serum and urinary creatinine and proteinuria were not different comparing untreated and telmisartan-treated SHRs. FC rats showed a continuous BP increase during the study while T rats reached the 15th week with a significantly low BP. The LV mass index was significantly smaller in the T group than in the FC group, as was the glomerular hypertrophy. The cardiomyocyte nuclei density per area and the cardiomyocyte mean cross-sectional area were smaller in the T group than in both the IC and FC groups. Intramyocardial artery densities (per area and per volume) were greater in the T group than in untreated SHRs, but myocardial fibrosis was reduced. In conclusion, telmisartan monotherapy effects on BP and also on the hypertension target organs, heart and kidney, are favorable. Telmisartan is able to attenuate SHR cardiomyocyte and glomerular hypertrophies, and myocardial reactive fibrosis as well. It also is favorable to the intramyocardial microcirculation.

Key-words: Telmisartan, Hypertension, Heart, Kidney, Stereology

Introduction

It is well known that hypertensive patients can greatly reduce the risk of cardiovascular events by controlling their blood pressure (BP) at normal levels. However, cardiovascular morbidity and mortality are associated with and preceded by organ damage that can be used as an intermediate endpoint for assessing the benefits of antihypertensive therapy (Zanchetti, 1999). Several orally-active non-peptide angiotensin II (Ang II) subtype 1 (AT1) receptor antagonists are now available for the treatment of hypertension. These drugs antagonize Ang II-induced biological actions, including smooth-muscle contraction, sympathetic pressor mechanisms, and aldosterone release (Bauer and Reams, 1995). These agents have a common mechanism of action - blockade of Ang II AT1 receptor - and their binding to this receptor is generally insurmountable (Burnier and Maillard, 2001); unlike angiotensin-converting enzyme inhibitors (ACEi), these agents neither inhibit bradykinin metabolism nor enhance prostaglandin synthesis (Kirk, 1999). Agents within this drug class differ in their pharmacological and pharmacokinetic properties, which may be translated into differences in the antihypertensive effect that may reflect in their clinical efficacy, especially at the end of the dosing interval (Burnier and Maillard, 2001; Fogari et al., 2002).

Hormonal factors, such as chronic inappropriate elevations in circulating Ang II and aldosterone, are accompanied by fibrosis of the heart's right and left sides. Haemodynamic factors regulate cardiomyocyte work and their adaptive hypertrophic growth. The relative contributions of hormonal and haemodynamic factors in regulating the growth of muscular and nonmuscular compartments must form the basis for the selection of pharmacological intervention that will optimize the management of symptomatic heart failure that accompanies hypertensive heart disease and ischemic cardiomyopathy. A regression of established cardiac fibrosis by its presumptive proteolytic digestion induced by ACEi or Ang II AT1 receptor antagonism has been demonstrated. This cardioreparative strategy improves the tissue stiffness, and suggests that diastolic dysfunction is reversible (Burlew and Weber, 2002).

Comparative clinical studies suggest that at recommended dose, losartan, which is the original drug in this class, has a lower antihypertensive efficacy than the newer agents, such as telmisartan. These differences between Ang II AT1 receptor antagonists are probably due to variations in the degree and duration of receptor

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blockade, and might be of clinical significance concerning the cardioprotective and renoprotective effects of this class of antihypertensive agents (Burnier and Maillard, 2001). The present study aims to evaluate the attenuating/regressing telmisartan effect on preexistent cardiac and renal lesions in mature SHRs.

Material and Methods

Fifteen adult male SHRs (one year old) were used in this study (from colonies maintained at the State University of Rio de Janeiro). The animals had body mass (BM) of 372±25 g (mean±SD) and blood pressure (BP) of 184±8 mmHg at the start of the study. BP was weekly verified in conscious rats through the non-invasive method of the tail-cuff plethysmography (Letica LE 5100, Panlab®). All animals were individually housed in a temperature- (21±1 ºC) and humidity-controlled (60±10%) room, submitted to a 12h-dark/light cycle and air exhaustion cycle (15min/h). Animals were housed during two weeks for acclimatization and trained to verify BP and BM with the minimum stress, and the daily water intake per animal was determined and used to dissolve drugs and to guarantee total intake of the planned daily drug dosage.

The SHRs were separated into three groups of five animals each and kept housed during 15 weeks. The groups were composed as follows: the initial control group (IC) - the SHRs received daily Nuvilab® food (Brazil) and fresh water ad libitum. BP and BM were verified in the first day of study, and they were then sacrificed; the final control group (FC) - the SHRs received food and fresh water ad libitum daily during the period of the study; and the telmisartan group (T) - the SHRs received food and 50 ml of fresh water containing 1.2 mg/kg of telmisartan daily (Boehringer Ingelhein, lote 203439).

All procedures were carried out in accordance with conventional guidelines for experimentation with animals (NIH Publication Nº. 85-23, revised 1996). The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation at the State University of Rio de Janeiro.

When the 15 weeks were over, animals were deeply anesthetized (intra-peritoneal sodium pentobarbital) and a cardiac injection of 10% KCl caused diastolic cardiac arrest. For urinary protein excretion measurement one day before the sacrifice, animals were kept in metabolic cages for 24 hours and the urine was collected. During this period, animals were not fed. Protein concentration was measured by the colorimetric method (Labtest Diagnostica®). Serum and urinary creatinine were determined by the alkaline picrate method (Labset kit) using the Mega Bayer automatic analyzer.

The heart and the left kidney were removed. The atria were separated from the ventricles and the left ventricle (including the interventricular septum) was separated from the right ventricle. All these portions were individually measured according to the liquid volume displacement method (Scherle, 1970). The heart mass/body mass ratio, the left ventricle mass/heart mass ratio, the left ventricle mass/body mass ratio (LV mass index), and the left kidney mass/body mass ratio were determined.

Stereology

The left ventricle and the left kidney were sectioned according to the orientator design (Mattfeldt et al., 1990a,b). The fragments were placed for 48h at room temperature in fixative (freshly prepared 4% w/v formaldehyde in 0.1M phosphate buffer, pH 7.2) (Carson et al., 1973) and then embedded in Paraplast plus® (Sigma, St Louis), sectioned at 3Ìm thickness, and stained with Sirius red or Masson trichrome. Five microscopic fields were analyzed per section, five sections per organ (five animals per group), totaling 125 fields per group. Counts used video-microscopy (Leica DMRBE microscope, Kappa video camera, Sony triniton monitor).

Myocardium

Different parameters were estimated using unbiased stereology (Gundersen et al., 1988; Cruz-Orive and Weibel, 1990; Mandarim-de-Lacerda, 1999). The myocardium was analyzed considering cardiac myocytes and cardiac interstitium (composed of connective tissue with nerves and vessels). A 3,600 μm^2 frame with 20 test points was used and considered the forbidden line (no structures crossing the right and inferior lines of the frame, or their extensions, were counted) (Gundersen, 1977).

Volume densities (V_V) of cardiomyocytes (cm), connective tissue (ct), and intramyocardial arteries (art) were estimated by point counting (V_V[structure]:= P_P[structure]/P_T, P_P is the number of points hitting the structure and P_T is the total number of test-points, := indicates that it is an estimate). Cardiomyocyte nuclei and intramyocardial artery density per area (Q_A[cm] or [art]) were estimated. The length density of the intramyocardial arteries was estimated (L_V[art]:= 2.Q_A[art]). The mean cross-sectional area (MCA) of cardiomyocyte and intramyocardial arteries was estimated (MCA[structure]:=V_V[structure]/2. Q_A[structure]).

Kidney

The mean volume-weighed glomerular volume (VWGV) was estimated through the “point-sampled” intercept method (Gundersen and Jensen, 1985). A test-system consisting of parallel lines associated with test points was superimposed on each field. The line direction on the sample was randomly determined. For each point inside the unbiased counting frame, which hits a glomerulus intercept through the point, measurement of the intercept length was performed

using a 32 mm long logarithmic rule composed of a series of 15 classes (Sorensen, 1989), and each individual intercept was cubed to obtain VWGV (Mandarim-de-Lacerda and Pereira, 2001; Mandarim-de-Lacerda et al., 2002; Pereira et al., 2002).

Statistical analysis

TJe differences in biometrical data were tested with one way analysis of variance and Newman-Keuls post-hoc test. The differences in the stereological data were tested by non-parametric Kruskal-Wallis analysis of variance and the Mann-Whitney test because these data are from non-normal discrete variables. The significance level of 0.05 was used for statistical significance (Zar, 1999).

Results

The Fig. 1 (BP), Fig. 2 (LV mass index), and Fig. 3 (VWGV) show the differences among the groups. No difference was seen in serum and urinary creatinine and proteinuria when comparing untreated and telmisartan-treated SHRs (Table 1). Table 2 shows the myocardium stereological indices.

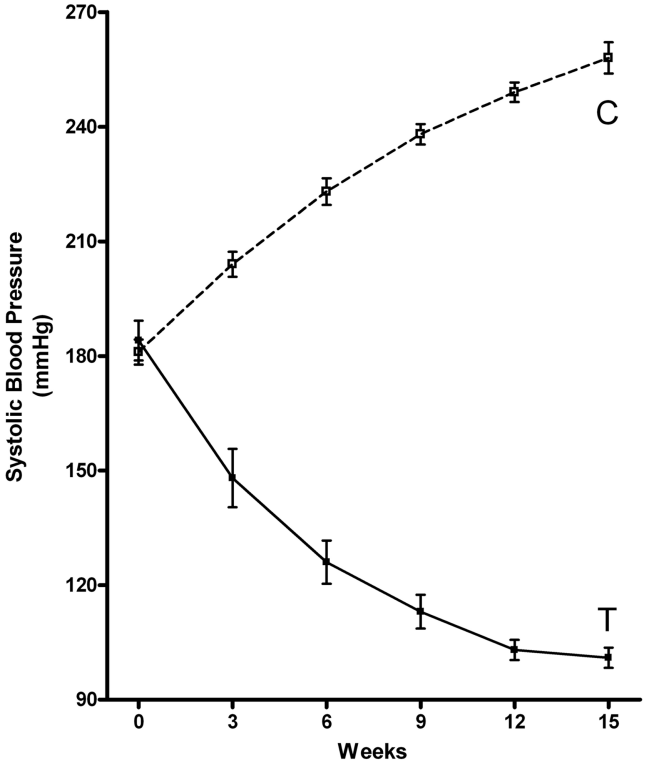

Fig. 1. Systolic blood pressure in control SHRs (C) and SHRs treated with telmisartan (T) during 15 weeks. Since week 3 the BP is significantly different between the groups.

Blood pressure and cardiac and glomerular hypertrophies

Control rats showed a continuous BP increase. During the study, BP reached (mean±SD) 258±12 mmHg in the 15th week (final control group). This tendency, observed in untreated SHRs, was greatly altered by telmisartan treatment and rats reached the 15th week with a significantly low BP (101±8 mmHg). The difference in the BP between untreated and treated SHRs was gradually more accentuated since the 3rd week until the end of the study (Fig. 1).

The glomerular hypertrophy relative to the ageing process in SHRs was efficiently reduced by the use of telmisartan. The LV mass index was significantly smaller in SHRs treated with telmisartan than in control SHRs (Fig. 2) but the left kidney mass/body mass ratio did not show difference between the groups. Glomerular hypertrophy was efficiently reduced as well, and animals reached the 15th week of the study with a smaller

Table 1. Serum and urinary creatinine levels and proteinuria (mean±SD) in final control and telmisartan groups. Differences are not significant between the two groups.

GROUPS	CREATININE (μmol/l)		PROTEINURIA (g/l)
	Serum	Urinary	
Final Control	70.4±6.2	8,906±5,210	74.6±18.7
Telmisartan	75.7±9.7	9,187±4,224	68.6±3.1

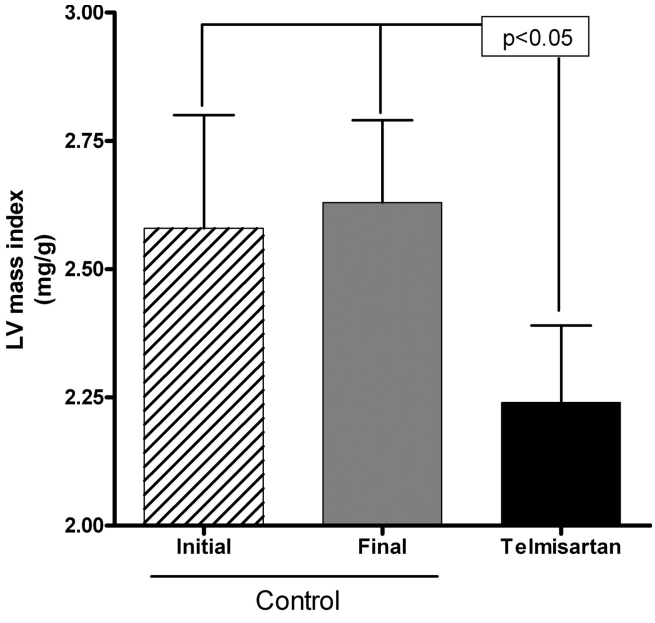

Fig. 2. LV mass index calculated in the initial and final control SHRs and in telmisartan-treated SHRs. Telmisartan SHRs showed significantly smaller LV mass index than control SHRs.

VWGV than the final control SHRs (Fig 3).

Myocardial remodeling

Cardiomyocytes

Despite the fact that the volume density of cardiomyocytes did not show any difference among the groups the cardiomyocyte nuclei density per area was roughly 40 per cent greater, and the cardiomyocyte mean cross-sectional area was roughly 30 per cent smaller in telmisartan rats than in the initial and final control SHRs.

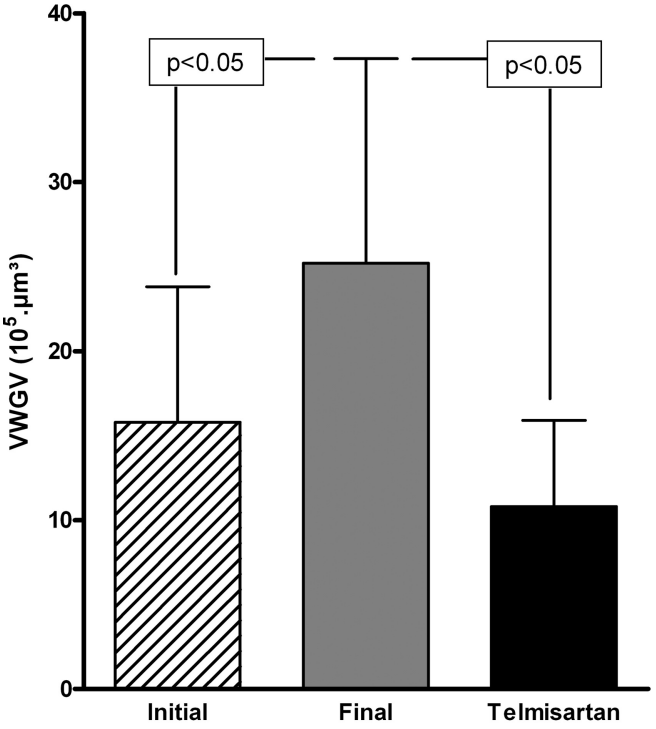

Fig. 3. Volume weighed glomerular volume (VWGV). A major VWGV observed in the final control SHR is compatible with glomerular hypertrophy in these animals but significantly different from initial control SHRs or telmisartan-treated SHRs.

Intramyocardial arteries

Intramyocardial artery densities (per area and per volume) were more than 50 per cent greater in telmisartan-treated SHRs than in untreated SHRs. As well as the intramyocardial arteries, length density was more than 20 per cent greater and the mean cross-sectional area was more than 15 per cent greater in telmisartan-treated SHRs than in the control ones. The artery/cardiomyocyte ratio, calculated as Vv[art]/Vv[cm], showed a ratio approximately 80 per cent greater in telmisartan-treated SHRs than in untreated SHRs.

Connective tissue

The quantification of the myocardial connective tissue allows us to explain the extension of the reactive myocardial fibrosis (interstitial and perivascular) occurring in response to the SHR cardiac overload. The telmisartan treatment reduced the myocardial fibrosis volume density in almost 40 per cent to the one observed in the final control SHRs. There was no difference in relation to the initial control ones.

Discussion

In this study, long-term telmisartan administration in mature SHRs favored BP control and attenuated left ventricular and glomerular hypertrophies. Furthermore, the analysis of telmisartan effects on myocardial remodeling also showed cardiac myocyte hypertrophy and reactive fibrosis attenuation, as well as improvement of all intramyocardial artery indices. These findings are important because SHRs normally develop cardiac and

Table 2. Stereology of the myocardium presented as mean (SD).

GROUPS	CARDIOMYOCITES			INTRAMYOCARDIAL ARTERIES				CONNECTIVE TISSUE
	Q_A 1/mm²	V_v %	MCA µm²	Q_A 1/mm²	V_v %	L_v µm/mm³	MCA µm²	V_v %
Control								
Initial	1260 (130)	72 (8)	2900 (500)	1,495 (100)	10 (3)	444 (37)	330 (70)	18 (7)
Final	1330 (70)	70 (4)	2600 (200)	1,540 (120)	10 (1.4)	430 (20)	320 (40)	21 (3)
Telmisartan	1830[a,b] (150)	70 (3)	1900[a,b] (200)	2,278[a,b] (250)	17[a,b] (1)	570[a,b] (38)	380[a,b] (30)	13[b] (3)

L_v: length density; MCA: mean cross-sectional area; Q_A: density per area; V_v: volume density. Mann-Whitney test in signaled cases, when compared, p<0.05, if: [a] when compared with Initial Control group, [b] with Final Control group.

renal lesions during their lives (Gerdes et al., 1996; Kost et al., 1996). Antihypertensive treatment only attenuates end-organ damage if it decreases BP. Moreover, if a given antihypertensive is effective, it sometimes even attenuates end-organ damage in nonhypotensive doses. On the other hand, some agents do decrease BP, but do not prevent end-organ damage (e.g. hydralazine in SHR) (Pinto et al., 1998). For the same level of attained BP and the same degree of BP reduction, Ang II AT1 receptor antagonists valsartan and losartan reduced urinary albumin excretion in patients with type 2 diabetes independent of the associated reduction in blood pressure (Viberti and Wheeldon, 2002; Zandbergen et al., 2003).

There is a chronic ischemic condition in cardiac hypertrophy due to cardiac overload (Burlew and Weber, 2002). Since 9 months old, it is common that SHRs show cardiac hypertrophy, marked myocardial fibrosis, activation of nonvascular interstitium, focal myocytial degeneration, capillarization reduction, and small intramyocardial artery microarteriopathy. Hypertrophy and hyperplasia of smooth muscle cells are involved in intramyocardial arterial growth processes in hypertensive heart remodeling (Amann et al., 1995). The present results suggest an efficient action of telmisartan in attenuating this condition in SHRs.

Ventricular remodeling is a process by which the size, the shape and the composition of cardiac chambers, as well as the thickness and composition of the walls are altered due to physical loads and/or receptor activation, whether created by loss or overload of cardiac myocytes, or the effects of external hormonal or chemical factors. Hypertrophy, dilation, myocyte loss, either due to necrosis or apoptosis, and myocyte hyperplasia are involved in this process (Sonnenblick and Anversa, 1999; Pessanha and Mandarim-de-Lacerda, 2000a). Continuous cell renewal in adult myocardium was thought to be impossible, but multipotent cardiac stem cells may be able to renew the myocardium and, under certain circumstances, can be coaxed to repair the injured heart. Methodologies are currently available to recognize and quantitatively measure the contribution of myocyte size, number, and death to the adaptation of the overloaded heart and its progression to cardiac failure (Anversa et al., 2002; Anversa and Nadal-Ginard, 2002). In this study, stereology was used to observe the myocardial component balance in untreated SHRs and SHRs treated with telmisartan. The major result in telmisartan-treated SHRs relative to cardiomyocytes was the remarkable cell size regression. Further studies are still necessary to analyze a possible myocardial cell turnover due to telmisartan treatment.

The heart has three-dimensional extracellular fibrillar collagen scaffolding that normally serves a variety of important functions for tissue integrity and efficiency of the muscular systolic pump and diastolic suction pump function. An adverse accumulation of extracellular matrix structural protein compromises the tissue stiffness, adversely affecting the myocardial viscoelasticity, and leads to ventricular diastolic and systolic dysfunction (Burlew and Weber, 2000). In NO-deficient rats, for example, the interstitial myocardial fibrosis augments and increases the animal morbidity and mortality (Pereira et al., 1998; Pechanova et al., 1999). In these hypertensive rats submitted to NO synthesis inhibition the myocardial healing process includes changes in extracellular matrix composition associated with phenotypic fibroblast modulation. Early and later lesion areas showed a population of spindle-shaped cells expressing alpha-smooth muscle actin content. These cells are apparently associated with type III collagen and fibronectin accumulation in the ischemic lesion areas contributing to the maintenance of the heart's mechanical performance throughout the healing process (Pessanha and Mandarim-de-Lacerda, 2000b). The Ang II AT1 receptor antagonism enhances basal NO availability and ameliorates vascular relaxations in SHRs (Montanari et al., 2002; Riveiro et al., 2002). Untreated mature SHRs commonly show an extensive reactive fibrosis, both interstitial and perivascular (Campbell et al., 1993). In the present study, telmisartan was efficient in reducing the SHR myocardial fibrosis.

All stereological indices showed differences among the groups, confirming the efficiency of the telmisartan treatment in preventing/attenuating myocardial vascular alterations usually seen in SHRs because of the rat ageing process. Intramyocardial artery densities were improved in telmisartan-treated SHRs. The artery/cardiomyocyte ratio was also greatly improved in telmisartan-treated SHRs than in untreated SHRs and probably it is not due to the regression of cardiac hypertrophy. This agrees with recent evidences of decreased microvessel density after myocardial infarct when the Ang II AT1 receptor is overexpressed that is amenable to AT1 receptor blockade, suggesting that efficacy of AT1 receptor blockers post-infarct may not only be due to attenuation of LV remodeling, but also to a stimulatory effect on angiogenesis (Boer et al., 2003).

In man, proteinuria is of clinical significance as a renal disease symptom. Rats, however, which are traditionally used for comparative investigations of renal physiology and pathology, have consistently demonstrated a striking physiological proteinuria. Albumin excretion increases significantly with age and it is associated with kidney disease, but the total protein excretion remains the same or even decreases slightly as the rat ages (Alt et al., 1980). The normal range of rat creatinine is wide, reflecting the variability due to strain, age, and sex differences (Sharp and La Regina, 1998). The present study was not able to demonstrate creatinine and proteinuria alterations in telmisartan-treated SHRs. The acute intravenous or subchronic oral administration of telmisartan to conscious dogs promotes diuresis and natriuresis without affecting potassium or creatinine excretion (Schierok et al., 2001). Likewise, neither a high-dose nor low-dose of perindopril had any effect on total renal filtration surface area; the observed beneficial

effects of ACE inhibition on kidney function are not the result of enhancement in glomerular capillary surface area (Dunstan et al., 2003). Compared with non-diabetic SHRs, untreated diabetic SHRs develop severe proteinuria and albuminuria. In diabetic SHRs, proteinuria and albuminuria are dose-dependent and are significantly attenuated by treatment with telmisartan and lisinopril. Telmisartan, but not lisinopril, significantly attenuates the diabetes-induced increase in glomerular volume (Wienen et al., 2001). The present results show less glomerular hypertrophy in telmisartan-treated SHRs than in the untreated SHRs, agreeing with a previous study that considered the renoprotective effect of telmisartan (Wagner et al., 1998).

The efficacy of ACEi therapy is well documented in chronic heart failure treatment (Brilla, 1994; Weber and Sun, 2000). Telmisartan was considered at least as effective as enalapril (Karlberg et al., 1999; Neutel et al., 2002), or lisinopril, causing less treatment-related angioedema than amlodipine (White, 2002) in treating elderly patients with mild to moderate hypertension, either as monotherapy or combined with other antihypertensive drugs (Freytag et al., 2002), and providing better long-term compliance and, consequently, better BP control than enalapril (Amerena et al., 2002).

In conclusion, the effects of telmisartan monotherapy on BP and also on the hypertension target organs, heart and kidney, are favorable. Telmisartan is able to attenuate SHR cardiomyocyte and glomerular hypertrophies, and myocardial reactive fibrosis as well. It also is favorable to the intramyocardial microcirculation.

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