Year-Long Antihypertensive Therapy With Candesartan Completely Prevents Development of Cardiovascular Organ Injuries in Spontaneously Hypertensive Rats

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Summary

Most previous studies have examined the effects of antihypertensive drugs in hypertensive animals for only a few months, and little information has been provided as to the protective effects of lifetime antihypertensive medication against cardiovascular organ injury. In this study, spontaneously hypertensive rats (SHR) were treated for 1 year with an angiotensin-II receptor antagonist (ARB) and the development of hypertensive organ injury was evaluated. Male 15-week-old SHR (n = 9) were given 25 mg/L candesartan (CS) in their drinking water for 1 year. Twelve SHR and 9 normotensive Wistar-Kyoto rats (WKY) were given normal tap water. Tail-cuff blood pressure was almost normalized by CS throughout 1 year (at 12-months: WKY 132 ± 3, SHR 229 ± 3, CS 137 ± 4 mmHg). After 1 year, cardiac ventricular weight (SHR +33%, CS -2% versus WKY) and aortic thickness (SHR +34%, CS +4% versus WKY) in the CS-treated SHR rats were not different than those of WKY. Echocardiographic midwall fractional shortening (SHR -18%, CS -1% versus WKY) and left ventricular hydroxyproline content (SHR +47%, CS +11% versus WKY) were also improved by CS to the WKY level. With respect to kidney function, GFR (SHR -24%, CS +9% versus WKY) was preserved, proteinuria (SHR +312%, CS +12% versus WKY) was reduced, and the histological glomerular injury rate (SHR +186%, CS +6% versus WKY) was reduced by CS. These results suggest that long-term antihypertensive therapy with CS can completely prevent hypertensive cardiovascular and renal injuries in SHR. (Int Heart J 2010; 51: 359-364)

Key words: Hypertension, Spontaneously hypertensive rats, Angiotensin II receptor antagonist, Left ventricular hypertrophy, Glomerular sclerosis

The ultimate goal of antihypertensive therapy is not only to normalize the blood pressure level but also to prevent end-organ damage, such as cardiac hypertrophy and renal dysfunction, and to prevent cardiovascular disease, such as stroke and myocardial infarction. Therefore, the efficacy of antihypertensive drugs on inhibition of hypertensive tissue injury and preservation of cardiovascular organ function is a matter of primary importance. Several studies have investigated the protective effects of antihypertensive drugs against cardiovascular organ injury using various animal models of hypertension, including mineralocorticoid-salt administration, renovascular hypertension, renal ablation and nitric oxide synthase inhibition, in which hypertensive organ injury progresses rapidly over a period of several weeks to months. However, this rapid temporal course of organ damage does not accurately reflect the organ damage that occurs over a span of decades in humans with essential hypertension. In this context, the pathophysiology of organ damage in spontaneously hypertensive rats (SHR) may more closely parallel that which occurs in humans with essential hypertension. SHR exhibit cardiac hypertrophy and arterial wall thickening with the development of hypertension. However, other types of organ injury, such as cardiac dysfunction and glomerular sclerosis, do not occur until a much later stage of life. Most previous studies using SHR have investigated the effects of antihypertensive therapy within a few months of administration, which may be inadequate to evaluate the cardiovascular organ protective effects of antihypertensive drugs, considering that the life span of SHR is approximately 20 months.

It has been suggested that the renin-angiotensin-aldosterone enhancement system (RAAS) is involved in the progression process of cardiovascular tissue and organ injury, and inhibitors of RAAS such as an angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor antagonist (ARB) are supposed to exhibit protective effects against the progression of cardiovascular organ injury beyond their hypotensive effects. The purpose of this study was to evaluate if lifelong antihypertensive therapy with an ARB can completely prevent the development of cardiovascular organ injury in SHR.

Methods

Treatment of rats: Male 15-week-old SHR (n = 21) and nor-
motensive Wistar-Kyoto rats (WKY, n = 9) were purchased from Charles River Japan (Atsugi, Kanagawa). Nine WKY and 12 SHR were fed standard chow and tap water, while 9 SHR were fed a standard chow and given water containing the ARB candesartan (CS, 25 mg/L). All animals were housed in a temperature- and light-controlled room. Systolic blood pressure was measured biweekly over a 12-month period using the tail-cuff method. At the end of the 12-month period, the rats were placed in metabolic cages for 24 hours for urine collection. The experiments were performed in accordance with the institutional guide for care and use of laboratory animals, and the study protocol was approved by our institutional animal research committee.

**Echocardiography:** After 12 months, transthoracic echocardiographic studies were performed under light anesthesia with intraperitoneal injection of ketamine HCl (10 mg/kg) and xylazine (10 mg/kg). Two-dimensional echocardiography and M-mode tracing were recorded at the level of the papillary muscles using a Toshiba (Tokyo) SSH-260A unit with a 7.0 MHz transducer placed on the shaved left hemithorax of the rats in the left decubitus position. M-mode measurements included left ventricular end-systolic and end-diastolic diameters (LVDs, LVDd), end-diastolic left ventricular posterior wall thickness (PWT), and interventricular septal thickness (IVST). Midwall fractional shortening (mFS) was calculated as follows:

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mFS = \left\{ \frac{LVDd + IVST/2 + PWT/2 - \{LVDd + IVST/2 + PWT/2\}^{1/3}}{LVDd + IVST/2 + PWT/2} \right\} / (LVDd + IVST/2 + PWT/2)
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The values of mFS were multiplied by 100 and expressed as % values.

Pulsed-wave Doppler spectra of mitral inflow velocities were recorded from the apical 4-chamber view with the sample volume placed near the tips of the mitral leaflets and adjusted to the position where the velocity was maximal and the flow patterns were laminar. The Doppler spectra were recorded on paper at 100 mm/s and analyzed off-line to determine peak early diastolic filling velocity (E) and peak filling velocity at atrial contraction (A). Measurements represent the mean of at least 3 consecutive cardiac cycles, and the E/A ratio as well as the deceleration time (DcT) of E wave was used for the evaluation of left ventricular diastolic function.

**Biochemical assay:** After performing the echocardiogram, blood samples were drawn from the inferior vena cava and centrifuged at 4°C to obtain serum. Serum and urinary concentrations of creatinine were measured by colorimetry using a commercial kit (Creatinine-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

A portion of the left ventricular free wall tissue was homogenized in 10 equivalent volumes of saline. A 0.5 mL aliquot of the homogenate was then mixed with 36% hydrochloric acid and heated to 100°C for 20 hours. Next, the mixture was centrifuged at 1,5000 g for 30 minutes, and a 0.1 mL aliquot of the supernatant was mixed with 1.5 mL of 0.3 N hydroxylithium. The hydroxyproline content in the reaction product was determined by high-performance liquid chromatography, and the value was expressed relative to tissue weight.

**Histological examination:** The brain, cardiac ventricles, descending thoracic aorta, and left kidney were excised and weighed. The right kidney was perfused and fixed with saline and neutral-buffered 8% formaldehyde solution. The right kidney and the upper half of the cardiac ventricles were embedded in paraffin, and 2-μm sections were cut for histological examination. These sections were stained with periodic acid-Schiff (PAS) and Masson trichrome. Histological examination was conducted in a blind manner. Fibrosis of the left ventricular wall was evaluated in sections stained with Masson trichrome. The area stained blue with aniline was quantified in 10 randomly-selected high-power fields (× 200) using a computer system (Image Quest, Hamamatsu Photonics; Hamamatsu and MacScope, Mitani Co., Fukui, Japan), and the average percent value was used for comparison. Glomerular lesions were evaluated in the juxtamedullary cortex, because juxtamedullary glomeruli are more vulnerable to sclerosis than glomeruli in the superficial layer of cortex. The glomerular injury rate (GIR) was assessed as the percentage of glomeruli showing glomerular sclerosis.

**Statistical analysis:** Values are expressed as the mean ± SE. Comparison of the 3 groups was performed by one-way ANOVA and post-hoc analysis using Dunnett’s multiple-range test. Time-course changes in parameters were analyzed by two-way ANOVA with post-hoc multiple comparisons using the Bonferroni-Dunn test. Non-parametric data were analyzed by the Kruskal-Wallis H-test followed by Tukey’s method for post-hoc between-group comparisons. A P value less than 0.05 was considered to indicate statistical significance.

**RESULTS**

**Physical measurements and organ weight:** All of the rats survived through the 12-month study period without presenting any signs of stroke or heart failure such as paresis and dyspnea. Figure 1 shows the time-course changes in body weight (upper panel), blood pressure (middle panel), and pulse rate (lower panel) during the one-year study period. The body weight increase was less in the untreated SHR than in the WKY, however, the difference was alleviated in the SHR treated with CS. SHR at 15 weeks of age before receiving CS (202 ± 3 mmHg) as well as the untreated control SHR (196 ± 4 mmHg) were hypertensive, while WKY were normotensive (135 ± 4 mmHg). The blood pressure of untreated SHR further increased in subsequent months, while WKY stayed almost normotensive throughout the study period. The systolic blood pressure of CS-treated SHR was maintained at a comparable level with WKY for 12 months. Regarding the pulse rate, untreated SHR showed a higher pulse rate than WKY at 4-months, 6-months, and thereafter. This increased pulse rate in SHR was alleviated by the CS treatment at 10-, 11- and 12-months.

The Table lists the body weight, systolic blood pressure, pulse rate, and cardiovascular organ weights of the rats at the end of the 12-month study period. As explained in Figure 1, body weight was lower, systolic blood pressure was much higher, and pulse rate was higher in untreated SHR than in WKY, however, these measurements were not significantly different between CS-treated SHR and WKY. The cardiac ventricular weight and the aortic weight were heavier in untreated SHR than in WKY, however, these increases were abolished by the one-year CS treatment. On the other hand, the weight of the brain or the kidney did not significantly differ between WKY, untreated SHR, and CS-treated SHR.

**Evaluation of cardiac injury:** Echocardiographic data obtained at the end of the 12-month study period are shown in the upper
The midwall fractional shortening, an index of left ventricular systolic function, was decreased in untreated SHR as compared with WKY, however, the one-year treatment with CS improved the value to a level comparable with that of WKY. The E/A ratio of transmitral flow velocity was significantly lower in untreated SHR than in WKY. This parameter of left ventricular diastolic function was also improved by the long-term CS treatment and the value was not significantly different between WKY and CS-treated SHR after the one-year study period. In agreement with this, the DcT, another parameter of diastolic function, showed values in agreement with E/A in WKY (48.5 ± 2.6 msec), untreated SHR (59.0 ± 3.7 msec, P < 0.05 versus WKY), and CS-treated SHR (46.6 ± 2.2 msec, P < 0.01 versus untreated SHR). The heart rates during echocardiographic measurements were not significantly different between WKY, untreated SHR, and CS-treated SHR (260 ± 4, 273 ± 10 and 261 ± 7 bpm, respectively).

The bar graphs in the lower panels of Figure 2 compare the extents of left ventricular wall tissue fibrosis between the 3 groups of rats. Findings of myocardial infarction were not observed even in untreated SHR. Either the histological staining of collagen or the chemical measurement of collagen amino acid content showed that the cardiac fibrosis was less marked in WKY and CS-treated SHR than in untreated SHR.

Evaluation of renal injury: The upper panels in Figure 3 show serum creatinine (left) and creatinine clearance (right) in the 3
cardial contractility, reduced glomerular sclerosis, and pre-
though blood pressure normalization was not achieved.\textsuperscript{420} According to the hyperfiltration theory, increases in glomerular capillary pressure, referred to as glomerular hypertension, play an important role in the progression of renal dysfunction.\textsuperscript{431} Intraglomerular capillary pressure is affected by the tone of the glomerular arterioles as well as by the level of systemic arterial pressure. Because angiotensin II is a potent constrictor of the efferent arterioles, ACE inhibitors and ARBs, which inhibit the generation and/or action of angiotensin II, are effective at alleviating glomerular hypertension.\textsuperscript{441} Multiple lines of clinical evidence have indicated that long-term inhibition of RAAS reduces proteinuria and attenuates the rate of deterioration of renal function in patients with diabetic and nondiabetic renal disease.\textsuperscript{445-450} However, in SHR or patients with essential hypertension, detectable signs of renal injury do not occur until the later stage of life,\textsuperscript{451} and it is not clear whether RAAS inhibitors can prevent or delay the onset of renal lesions in patients without pre-existing renal disease. In this respect, the present study demonstrated that lifelong antihypertensive therapy with an ARB can preserve renal function as indicated by creatinine clearance in SHR. Since the tubular excretion of creatinine is not negligible in rats, the observed changes in creatinine clearance may not accurately reflect the actual changes in glomerular filtration rate. However, considering that the ARB also prevented the development of proteinuria and glomerular sclerosis in SHR to comparable levels as that of WKY, the long-term treatment with an ARB is thought to provide renoprotective effects in SHR.

It is widely recognized that RAAS is involved in the pathogenesis of hypertension, and recent clinical trials in which RAAS inhibitors were administered to subjects with high normal blood pressure have shown an ARB or an ACE inhibitor can reduce later development of hypertension in these prehypertensive subjects.\textsuperscript{40,450} In addition to this, if the results of our current study are applicable to humans, lifelong ARB treatment starting at an early age in subjects with a genetic predisposition to hypertension would be expected to prevent blood pressure elevation and the development of cardiovascular organ injury throughout life.

In conclusion, the present study has demonstrated that lifelong treatment of hypertension with the ARB candesartan can completely prevent the development of left ventricular hypertrophy, myocardial fibrosis, cardiac dysfunction, glomerular sclerosis, and renal dysfunction in SHR as well as normalizing blood pressure. If these effects can be extended to clinical use in humans, the preventive use of RAAS inhibitors from the prehypertensive or an early stage of essential hypertension may reduce the incidence of long-term cardiovascular organ injury and cardiovascular diseases later on in life.

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**References**


22. Komatsu K, Frohlich ED, Ono H, Ono Y, Numabe A, Willis GW. Glomerular dynamics and morphology of aged spontaneously hy-