Effects of Long-Term Treatment with Candesartan on Hemodynamics and Organ Damage in Spontaneously Hypertensive Rats

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Summary. This study was designed to investigate the effects of candesartan on blood pressure (BP) and blood pressure variability (BPV) reductions, baroreflex sensitivity (BRS) amelioration, and organ protection in spontaneously hypertensive rats (SHR). Methods: Studies were performed in two groups of SHR (n = 13 for control rats; and n = 20 for candesartan-treated rats) and one group of WKY rats (n = 13). Candesartan (3mg/kg/d) was given in rat chow for 4 months. BP was then continuously recorded for 24 hours in conscious state. After the determination of BRS, rats were killed for organ-damage evaluation. Results: Long-term treatment with candesartan significantly reduced BP and BPV expressed by both standard deviation and variation coefficient of BP, enhanced BRS and produced obvious organ protection. Compared with BP level, BPV and BRS values showed a closer or similar relationship with organ-damage parameters in SHR. Multiple regression analysis showed that the decrease in left ventricular hypertrophy was most closely associated with the increase in BRS, whereas the decrease in aortic hypertrophy was most closely associated with the decrease in 24-hour systolic BPV, and the amelioration in renal lesions, with the increase in BRS and the decrease in 24-hour systolic BPV. Conclusion: long-term treatment with candesartan results in organ protection in SHR. Besides BP reduction, the decrease in BPV and the restoration of BRS are significantly related to this organ protection.

Key Words: Hypertension, Candesartan, Organ damage, Blood pressure variability, Baroreflex sensitivity

Introduction

It has been demonstrated that the organ protective effects of AT1-receptor blockers in hypertension are mainly derived from hemodynamic mechanisms, such as a substantial reduction in blood pressure (BP) level and non-hemodynamic mechanisms, such as, among others, cellular growth inhibition [1–3]. Among the hemodynamic mechanisms studied, the association between a reduction in BP and a reduction in organ damage in humans is now well established [4]. However, BP is certainly not the only hemodynamic determinant for organ damage. Recently, it has been proposed that blood pressure variability (BPV) and baroreflex sensitivity (BRS) may be two other important hemodynamic factors associated with organ damage in hypertension [5–10]. Candesartan cilexetil (candesartan) is an AT1 receptor antagonist with a long duration of hypotensive action [11]. However, up to date, no information is available about the role of BPV and BRS in the organ protective effects of candesartan during long-term treatment. Accordingly, the present work was designed to investigate the effects of long-term treatment with candesartan on BP, BPV, BRS and organ damage and to elucidate the importance of BPV reduction and BRS amelioration in organ protection in spontaneously hypertensive rats (SHR) treated with candesartan.

Methods

Animals

Male SHR and Wistar-Kyoto (WKY) rats aged 18 weeks were provided by the animal center of our university. The rats were housed under controlled temperature (23–25°C) and lighting (8:00–20:00 light, 20:00–8:00 dark) and with free access to food and tap water. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

Drug administration

Studies were performed in two groups of SHR (n = 13 for control rats; and n = 20 for candesartan-treated rats) and one group of WKY rats (n = 13). Candesartan (AstraZeneca, Molndal, Sweden) was mixed in the rat chow. The consumption of rat chow containing drugs was determined previously. The content of drugs in the rat chow was calculated according to the chow consumption.
consumption, and the ingested dose of drug was approximately 3mg/kg/d. The control SHR group and WKY rats received the same diet without the drugs. After 4 months of drug administration, BP was recorded during 24 hours, and then BPV was calculated and BRS determined in conscious, freely moving rats. Histopathological examinations were performed after BP recording and BRS studies.

**BP measurement**

Systolic BP (SBP), diastolic BP (DBP) and heart period (HP) of rats were continuously recorded using a previously described technique [12, 13]. Briefly, rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A floating polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter was placed into the left femoral vein for intravenous injection. The catheters were exteriorized through the interscapular skin. After a 3-day recovery period, the animals were placed for BP recording in individual cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer (PT14M2, Fu-Dan Univ., Shanghai, China) via a rotating swivel that allowed the animals to move freely in the cage. After about 14-h habituation, the BP signal was digitized by a microcomputer. SBP, DBP and HP values from every heartbeat were determined on line. The mean values and standard deviation (SD) of these parameters during a period of 24 hours were calculated. The SD was defined as the quantitative parameter of BPV, i.e. systolic BPV (SBPV), diastolic BPV (DBPV), and HP variability (HPV). The coefficient of variation (CV) of BP (CV = SD/mean BP value) was taken as the measure of normalized BPV, i.e. CV for SBP (SBPV-CV) and CV for DBP (DBPV-CV). In addition, these parameters were also calculated for daytime (8:00–20:00) and nighttime (20:00–8:00) respectively.

**BRS measurement**

To determine the function of arterial baroreflex in conscious rats, the methods widely used are derived from that of Smyth firstly applied for humans [14]. The principle of this method is to measure the prolongation of HP in response to an elevation of BP. With some modifications, this method was used in conscious rats [15, 16]. A bolus injection of phenylephrine was used to induce an elevation of BP. The dose of phenylephrine was adjusted to raise SBP between 20 and 40 mmHg. HP was plotted against SBP for linear regression analysis and the slope of SBP-HP was expressed as BRS (ms/mmHg). BRS was assessed twice by phenylephrine injection in the same animals, and the mean value of the two assessments was denoted as BRS of the animals. The interval between two injections was more than 15 minutes.

**Morphological examination**

Morphological examinations were performed after BP recording and BRS studies. The animals were weighed and killed by decapitation. The thoracic and peritoneal cavities were immediately opened. The right kidney, aorta and heart were excised and rinsed in cold physiological saline. The right kidney was blotted. The left ventricle was isolated, blotted, and weighed. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 30-mm-long segment of thoracic aorta was harvested, blotted, and weighed. Ratios of left ventricular weight to body weight (LVW/BW) and aortic weight to the length of aorta (AW/length) were calculated [17–19]. Histopathological observation was also carried out with our conventional method [20]. Briefly, immediately after gross detection, all samples of kidneys were immersed in formalin solution for more than 1 week, dehydrated in ethanol, cleared in dimethylbenzene and embedded in paraffin. Then the 5-μm-thick sections were prepared and stained with hematoxylin and eosin for light microscopic evaluation.

**Glomerulosclerosis score**

For the semiquantitative evaluation of glomerular damage, the glomerulosclerosis score (GSS) was defined as previously described [21]. On the light microscopic specimens, approximately 50 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded according to the degree of sclerosis: 0, if no mesangial expansion; 1, if mild mesangial expansion (less than 30% of a glomerular area); 2, if moderate mesangial expansion (30–60% of a glomerular area); 3, if marked mesangial expansion (more than 60% of a glomerular area); and 4, if the sclerosis was global. This was performed by one observer in a blind fashion using coded slides. A weighted composite sclerosis score was then calculated for each kidney according to the following formula: glomerulosclerosis score = (1 × (number of grade 1 glomeruli) + 2 × (number of grade 2 glomeruli) + 3 × (number of grade 3 glomeruli) + 4 × (number of grade 4 glomeruli)) × 100/(number of glomeruli observed).

**Statistical analysis**

Data are expressed as mean±SEM. Comparisons among groups were made by ANOVA followed by Duncan test. The relationships between hemodynamic parameters and organ damage parameters were analyzed by classic univariate correlation analysis. Stepwise multiple-regression analysis was performed to study the independent effect of hemodynamic parameters on organ damage. F to enter and F to remove were set to P < 0.05 and P > 0.10 respectively. P < 0.05 was considered statistically significant. Statistical analysis was performed by using software SPSS 11.0.0.