Systemic Effects of Carteolol, a β-Adrenoceptor Antagonist in Stroke-Prone Spontaneously Hypertensive Rats

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The preventive effects of carteolol, a β-adrenoceptor antagonist, on secondary lesions were pathophysiologically examined in stroke-prone spontaneously hypertensive rats (SHRSP) from 8 to 30 weeks of age. Carteolol was added to the drinking water in doses of 0.005% (8 to 18 weeks of age) to 0.01% (19 to 30 weeks of age) (3.8 and 6.0 mg/kg/d, respectively). These animals gained significantly more weight than the untreated control SHRSP, and their heart rate was reduced from 14 weeks of age. Suppression of blood pressure rise was not definite, however, histology revealed prevention of the development or aggravation of secondary hypertension-related lesions, such as myocardial fibrosis, proliferative arteriolitis, necrotic arteriolitis and renal glomerular lesions. A decrease in non-esterified fatty acids in the serum was evident. Thus, carteolol has cardiac as well as renal protective effects, in the SHRSP.

Keywords — carteolol; β-adrenoceptor antagonist; stroke-prone spontaneously hypertensive rat; antihypertensive; secondary hypertensive lesion

Introduction

Carteolol, synthesized by Nakagawa et al. 1) is a potent β-adrenoceptor antagonist widely prescribed for patients as an anti-arrhythmic, anti-anginal and antihypertensive agent. This drug has non-cardioselective β-blocking ability, and weak central and intrinsic sympathomimetic actions (ISA). 2) Studies on hypertensive patients showed that the drug was more potent than propranolol at about 1/5 the oral dose, it sustains both systolic and diastolic pressures at lower levels, without any marked reduction in heart rate as the result of intrinsic sympathomimetic actions. 3) We reported the antihypertensive effects of several β-adrenoceptor antagonists, on spontaneously hypertensive rats (SHR) and on stroke-prone spontaneously hypertensive rats (SHRSP). We obtained data that drugs in this class were not only effective in controlling blood pressure but also protected against secondary untoward events related to hypertension. 4)–8) The present study was done to investigate the chronic effects of carteolol on SHRSP.

Materials and Methods

Male SHRSP and Wistar Kyoto rats (WKY) maintained and bred at the Animal Laboratory Center of the Nagasaki University School of Medicine were used. All these animals were fed a standard diet (F-2, Funabashi Farm Co., Chiba, Japan) and water ad libitum, and housed at 24 °C, with lights on from 0600 to 1800 h. Of 22 male SHRSP at 8 weeks of age, 11 rats were given drinking water containing carteolol in a dose of 0.005% (w/v) from 8 to 18 weeks of age and 0.01% (w/v) from 19 to 30 weeks of age. The remaining animals given no drug served as the controls. The method used to calculate the amount of the drug ingested was to measure the daily intake of drinking water which contained the drug. There were no differences in water intake between control and carteolol-treated rats. The mean value of carteolol intake was calculated to be 3.8 and 6.0 mg/kg/d, respectively. Systolic blood pressure was measured indirectly using an electrosphygmomanometer (PE-300, Narco-Biosystem Co., U.S.A.) after prewarming

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the tail for 10 min at 38 °C to dilate the caudal artery. Body weight was measured once a week, and blood pressure and heart rate once in the morning (0900 to 1100 h) every other week throughout the experimental period. At termination of the study, the animals were fasted overnight then killed. Blood sampling and analysis, organ weight measurement and histological examinations were carried out. Serum samples were prepared and blood urea nitrogen, creatinine, triglyceride, non-esterified fatty acids, total cholesterol and phospholipid levels were measured using reported methods. The brain, heart, lungs, kidneys, liver, spleen, adrenal glands, aorta and mesenteric arteries were weighed and tissue specimens were prepared for histopathological examination. All the values obtained were statistically evaluated by the one-way analysis of variance (ANOVA) using the F-test, at a $p$ value of less than 0.05.

Results

Body Weight
While the SHRSP do not gain weight as do the normotensive WKY, the SHRSP treated with carteolol were significantly heavier from 15 weeks of age onwards, as compared to the matching controls (Fig. 1).

Systolic Blood Pressure
Initial measurements showed that the systolic blood pressure in both treated and control groups was around 182 mmHg. At the beginning, the 10 and 12-week-treated group showed a significantly lower blood pressure than did the control group, then the level continued to rise throughout 22 weeks of treatment, in both groups (Fig. 2).

Heart Rate
The initial rates of heart beat were around 380 beats/min in the treated and control groups. Treatment with carteolol significantly reduced

Fig. 1. Body Weight of Male SHRSP from 8 to 30 Weeks of Age
Values are means ± S.E. of 11 animals (●) treated with carteolol (3.8 and 6.0 mg/kg/d from 8 to 19 and 30 weeks of age, respectively) in drinking water and eleven controls (○) treated with water alone. $a) p < 0.05$, $b) p < 0.01$.

Fig. 2. Systolic Blood Pressure of Male SHRSP from 8 to 30 Weeks of Age
Values are means ± S.E. of 11 animals (●) treated with carteolol (3.8 and 6.0 mg/kg/d from 8 to 19 and 19 to 30 weeks of age, respectively) in drinking water and eleven controls (○) treated with water alone. $a) p < 0.05$, $b) p < 0.01$.

Fig. 3. Heart rate of Male SHRSP from 8 to 30 Weeks of Age
Values are means ± S.E. of 11 animals (●) treated with carteolol (3.8 and 6.0 mg/kg/d from 8 to 19 and 19 to 30 weeks of age, respectively) in drinking water and eleven controls (○) treated with water alone. $a) p < 0.05$, $b) p < 0.01$. 
TABLE I. Changes in Serum Levels of Blood Urea Nitrogen, Creatine and Lipids of SHRSP Treated or not Treated with Car-teolol

<table>
<thead>
<tr>
<th></th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>NEFA (mEq/l)</th>
<th>TC (mg/dl)</th>
<th>PL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SHRSP (11)</td>
<td>20.8 ± 1.88</td>
<td>0.58 ± 0.05</td>
<td>41.5 ± 3.09</td>
<td>987.0 ± 94.5</td>
<td>63.3 ± 3.10</td>
<td>144.7 ± 5.76</td>
</tr>
<tr>
<td>Treated SHRSP (11)</td>
<td>18.7 ± 0.84</td>
<td>0.58 ± 0.04</td>
<td>33.5 ± 2.29</td>
<td>725.5 ± 67.7 (^a)</td>
<td>66.5 ± 2.25</td>
<td>160.7 ± 8.58</td>
</tr>
</tbody>
</table>

Values are means ± S.E. Treatment consisted of carteolol in doses of 3.8 and 6.0 mg/kg/d added to the drinking water of rats aged from 8 to 19 and 19 to 30 weeks, respectively. Water alone was given to the controls. Number of rats is given in parentheses. \(^a\) \( p < 0.05 \) compared with the controls. BUN, blood urea nitrogen; Cr, creatinine; TG, triglyceride; NEFA, non-esterified fatty acids; TC, total cholesterol; PL, phospholipid.

TABLE II. Organ-body Weight Ratios

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Liver</th>
<th>Adrenal ((\times 10^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SHRSP (11)</td>
<td>6.53 ± 0.15</td>
<td>6.08 ± 0.28</td>
<td>5.09 ± 0.29</td>
<td>7.69 ± 0.11</td>
<td>1.99 ± 0.14</td>
<td>28.6 ± 0.53</td>
<td>1.67 ± 0.20</td>
</tr>
<tr>
<td>Treated SHRSP (11)</td>
<td>5.97 ± 0.11 (^b)</td>
<td>6.78 ± 0.22</td>
<td>4.26 ± 0.17 (^a)</td>
<td>7.14 ± 0.13 (^b)</td>
<td>1.75 ± 0.04</td>
<td>25.3 ± 0.23 (^b)</td>
<td>1.28 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± S.E. of weight (mg)/body weight (g). Number of rats is given in parentheses. \(^a\) \( p < 0.05 \) and \(^b\) \( p < 0.01 \), compared with each control.

The rate of 385 to 320 beats/min, from 14 to 30 weeks of age (Fig. 3). The control value at the end of treatment was 445 beats/min.

**Serum Biochemistry**

Among the biochemical parameters, carteolol treatment significantly lowered the serum level of non-esterified fatty acids (Table I). The triglyceride and blood urea nitrogen were also reduced, albeit not significantly.

**Organ Weights**

![Fig. 4. (A) Marked Perivascular and Myocardial Fibrosis (★) in the Heart of the Control SHRSP](image)
Arrows indicate coronary arteries. × 200 (B) Slight perivascular fibrosis in the heart of SHRSP treated with carteolol for 22 weeks. × 400 Specimens were stained with Azan-Mallory.
The brain, heart, lungs, kidneys, liver, spleen and adrenal glands in the treated animals were weighed and the findings compared with those in the respective control organs (Table II). There were significant decreases in weight of the brain, heart, kidney and liver, in the carteolol administered rats.

**Histopathological Findings**

In one of the eleven control animals, a brain lesion developed. In no SHRSP treated with carteolol was there a pathological lesion in the brain.

The heart was transversely dissected from the septum to the right and left ventricles. Pronounced fibrosis of cardiac muscle fibers was noted in one control animal with coronary arteriolitis. Fibrosis occurred in other treated and control animals, but all were mild or moderate, and comparable in incidence (Fig. 4). When the thickness of the left ventricular wall was measured with calipers, the carteolol treated animals showed a significant reduction in adaptive hypertrophy ($2.34 \pm 0.03$ mm vs. $2.61 \pm 0.05$ mm in the control, $p < 0.01$).

The kidneys were dissected to include the hilum. Care was taken to search for proliferative and necrotic arteriolitis, and glomerular changes (Fig. 5). The number of these lesions was noted and converted to 100-fold magnification. As shown in Table III, renal lesions were fewer in number in the carteolol treated animals.

The abdominal aorta was histologically examined. Arteriolitis was not present in either the treated or the control animals. However, the arterial media of the control animals were significantly thicker than those of the treated animals ($130 \pm 3.4 \mu m$ vs. $108.7 \pm 2.1 \mu m$, $p < 0.01$).

The mesentery was dissected out perpendicular to the mesenteric arteries to observe as many arteries as possible. Neither group showed signs

<table>
<thead>
<tr>
<th>TABLE III. Histopathological Lesions in the Kidneys of SHRSP Treated with Carteolol</th>
<th>Controls</th>
<th>Treated animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative arteriolitis</td>
<td>$0.19 \pm 0.05$</td>
<td>$0.01 \pm 0.01 \ b)$</td>
</tr>
<tr>
<td>Necrotic arteriolitis</td>
<td>$0.08 \pm 0.03$</td>
<td>$0 \ a)$</td>
</tr>
<tr>
<td>Glomerular lesions</td>
<td>$0.13 \pm 0.04$</td>
<td>$0.01 \pm 0.01 \ a)$</td>
</tr>
</tbody>
</table>

Values are expressed as number per $\times 40$ high power field (means $\pm$ S.E. of 11 animals each). Treatment consisted of carteolol at 3.8 and 6.0 mg/kg/d from 8 to 18 and 19 to 30 weeks of age, respectively, given in the drinking water. Controls received water alone. $a) p < 0.05$ and $b) p < 0.01$ compared with each control.
of arteriolitis, however, in one control animal, there was a pronounced lymphocytic infiltration, probably due to ascites retention.

Adrenal glands were dissected in the central portion to include both the cortex and medulla. Thrombosis was formed in the capsular artery in two of eleven control animals, and a cortical nodule was formed in five of ten treated animals and eight of eleven control animals. The adrenal medulla appeared morphometrically normal, but the zona glomerulosa of the treated animals was thicker than that of the control animals (47.73 ± 3.29 μm vs. 60.92 ± 3.99 μm, p < 0.01).

The lungs, liver and spleen showed no specific lesions, though a slight congestion was sometimes evident.

Discussion

We reported that propranolol and pindolol given in drinking water at concentrations of 0.025% and 0.005%, respectively, are effective treatments for the SHRSP. The β-adrenergic blocking potency of carteolol was determined using isoproterenol and cardiac nerve stimulation. Since it was found to be 20–30 times stronger than that of propranolol and comparable to that of pindolol, carteolol was added to the drinking water in doses of 0.005% and 0.01%. We examined the clinical effects of carteolol, a long-acting β-adrenoceptor antagonist, in the treatment of SHRSP.

The animals treated with carteolol gained more weight than did the untreated controls during the total 22 week-treatment period. It is noteworthy that the hypotensive effects of carteolol were weak in doses of 3.8 and 6.0 mg/kg/d during the respective treatment periods of 11 weeks (8 to 19 weeks of age) and 11 weeks (19 to 30 weeks of age). The depressor effect of carteolol was apparent only in animals at 10 and 12 weeks of age. Histological examinations confirmed that carteolol had appreciable and beneficial effects in preventing or reducing the incidence of secondary hypertensive lesions common to SHRSP, such as renal lesions, cardiac hypertrophy and thickening of the arterial wall. We and others obtained evidence for the prophylactic effect of arotinolol, a β-adrenoceptor antagonist, and condensed tannins on the cerebrovascular damage in the SHRSP, in the absence of any decrease in blood pressure. Hence, the effects of carteolol on other factors linked to the pathophysiology of hypertensive complications have to be considered. The possibility that these preventive effects are due to the hypotensive effect of the drug cannot be ruled out. The indirect method of blood pressure measurement we used might not be so sensitive, and in addition, measurement in the a.m. might not be appropriate for determining the hypotensive effect due to nocturnal habits and high systolic blood pressure of rats in the night. Much remains to be learned about the role of pharmacological activity in the prevention of secondary hypertensive lesions.

The sympathetic nerve is usually hyperactive in the SHRSP. The reduction in heart rate in the SHRSP to near normal after 14 weeks of age was considered to indicate that the drug manifested a potent β-blocking action.

Renal lesions were less extensive in the treated than in the untreated SHRSP. The drug protected against renal lesions in this model, and this effect seemed to be further evidenced by prevention of thickening of the cortical zona glomerulosa in the adrenal glands. The renin-angiotensin system is also active in SHRSP, probably due to renal vascular changes as well as central factors involved in the genesis of hypertension. If such is indeed the case, then the preventive effect of the drug on the zona glomerulosa would lead to an amelioration of renal lesions, in the presence of hypertension. Carteolol, unlike other β-adrenoceptor antagonists, also inhibited increases in serum levels of triglyceride. As carteolol was found to have cardiac as well as renal protective effects in the SHRSP, this drug warrants further attention for the possible prevention of secondary lesions in renal vascular systems.

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References


