A Study of Effect of CS-045, a New Antidiabetic Drug, on Hypertension in Spontaneously Hypertensive Rat

Mikiko KAWAGUCHI, Keiichiro TANIGAWA* and Yuzuru KATO*

Shimane Nursing College and *Department of Internal Medicine, Shimane Medical University, Izumo 693, Japan

Abstract. Several lines of evidence have suggested that insulin resistance may play an important role in the pathogenesis of hypertension. CS-045 is a new hypoglycemic drug by which improves insulin sensitivity in peripheral tissues. We assessed the effect of CS-045 on hypertension in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) as a control. At 20 weeks of age, the treatment with CS-045 was started by being mixed with chow pellets in the proportion of 0.2% (w/w) and continued for 6 weeks. Nonfasting plasma glucose levels were not changed in either strain of rats treated with CS-045, but plasma insulin levels decreased in SHR 4 and 5 weeks after the start of CS-045 therapy. Blood pressure increased with age in SHR without treatment, but CS-045 decreased blood pressure only 4 and 6 weeks after the treatment. These findings suggest that insulin resistance is not strongly associated with the pathogenesis of hypertension in SHR.

Key words: Hypertension, Insulin resistance, CS-045, Spontaneously hypertensive rats

THE PATHOGENESIS of essential hypertension remains to be fully investigated. It has been suggested that increased insulin resistance may be responsible for hypertension [1-3]. To assess the relationship between insulin resistance and hypertension, spontaneously hypertensive rats (SHR) have been employed as an animal model. Previous studies [4, 5] demonstrated impaired insulin sensitivity in SHR by means of a glucose clamp method compared with normotensive Wistar-Kyoto rats (WKY), but others [6] did not confirm these findings. The precise nature of the relationship between insulin resistance and hypertension in SHR therefore remains to be elucidated.

CS-045 is a new thiazolidinedione hypoglycemic agent, of which the effect is attributed to a decrease in insulin resistance in the liver and other peripheral tissues [7-9]. Fujiwara et al. [10] found that CS-045 improved hyperglycemia and hyperinsulinemia in the KK mouse, the ob/ob mouse and the Zucker fatty rat, animal models of insulin-resistant diabetes. In the present study, we assessed whether CS-045 is effective in ameliorating hypertension in SHR.

Materials and Methods

Male SHR and WKY were purchased from Charles River Laboratories (Atsugi, Japan) and bled in our animal facility. The animals were housed in air-conditioned quarters at 24 °C under artificial lighting (lights on 0800-2000 h). Liberal quantities of tap water and chow pellets (Funabashi Farm, Chiba, Japan) were provided.

At the age of 20 weeks, both SHR and WKY were divided into two groups. CS-045 (Sankyo Pharmaceutical Co., Tokyo) was given p.o. after being mixed with chow pellets in the proportion of 0.2% (w/w). The approximate dose of CS-045...
was 200 mg/kg/day. This dose of CS-045 effectively ameliorated hyperglycemia and hyperinsulinemia in insulin-resistant diabetic animals [10, 11]. The nonfasting rats were weighed weekly and blood samples were obtained by snipping the tail. Systolic blood pressure was measured weekly by means of the tail-cuff (automatic blood pressure recorder UR-1000 type, Ueda Manufacturing, Tokyo) after prewarming it to 37 °C for 10 min. The average of five readings was taken.

At the end of treatment, intravenous glucose tolerance test (IVGTT, 0.5 g/ml/kg BW) was performed as described previously [12]. Food was removed at 0900 h on days when measurements were taken, and all procedures were initiated 5 h later as indicated. Rats were anesthetized by i.p. injection of pentobarbital (50 mg/kg BW), and then glucose (50% solution) was injected into the saphenous vein. Blood samples were obtained immediately before, and 5, 10, 15 and 30 min after glucose administration.

Plasma glucose was measured with a glucose analyzer (Fuji Film Co., Tokyo, Japan). Immunoreactive insulin was measured by specific radioimmunoassay [13] with rat insulin as the standard.

The data are expressed as the means ± SEM. Statistical significance was determined by one-way analysis of variance followed by Fisher’s multiple-comparison test. P<0.05 was considered significant.

Results

At 20 weeks of age, there were no differences in body weight between SHR (355 ± 4 g, n=10) and WKY (360 ± 10 g, n=12). Until 24 weeks of age, body weight gain was obtained weekly. After that period, body weight remained stable and there were no differences between SHR and WKY with or without treatment.

Figure 1 illustrates the changes in fed plasma glucose (upper panel) and insulin (lower panel) levels in the experimental animals for 6 weeks. At 20 weeks of age, plasma glucose levels were lower in SHR than in WKY (138.2 ± 3.6 vs. 156.8 ± 4.5 mg/dl, P<0.01). CS-045 did not affect plasma glucose levels in either WKY or SHR. There were no differences between the untreated WKY and the untreated SHR in plasma insulin concentrations. CS-045 treatment resulted in a significant decrease in plasma insulin levels in the WKY (3.6 ± 0.2 vs. 2.2 ± 0.1 ng/ml, P<0.05) 4 weeks after treatment. Plasma insulin concentrations were significantly (P<0.05) lower in the SHR treated with CS-045 than in the untreated SHR at 4 weeks (4.0 ± 1.4 vs. 1.8 ± 0.2 ng/ml) and 5 weeks (4.6 ± 1.4 vs. 2.1 ± 0.3 ng/ml) after the start of experiments.

The changes in blood pressure during the 6 weeks are shown in Fig. 2. Before the start of treatment, blood pressure was much greater in SHR
EFFECT OF CS-045 ON HYPERTENSION IN SHR

559

than in WKY (231 ± 9 vs. 169 ± 3 mm Hg, P<0.001). Blood pressure further increased in the SHR during the experimental period. CS-045 treatment lowered blood pressure in the SHR. Statistical significance (P<0.05) was only obtained 4 weeks (221 ± 6 vs. 246 ± 6 mm Hg) and 6 weeks (222 ± 6 vs. 258 ± 3 mm Hg) after the start of treatment.

Figure 3 shows the results of IVGTT performed at the end of the experiment for 6 weeks. Fasting glucose levels were higher in the untreated WKY than in other rats. The peak of plasma glucose levels obtained 5 min after glucose load was greater in the untreated WKY than in the untreated SHR (368 ± 32 vs. 269 ± 20 mg/dl, P<0.05). Plasma glucose levels were higher in the untreated WKY than in the untreated SHR 10 min (P<0.05), 15 min (P<0.0005) and 30 min (P<0.0001) after glucose injection. Similarly, the WKY treated with CS-045 had higher plasma glucose levels than the treated SHR 15 min (P<0.05) and 30 min (P<0.01) after glucose load.

After 5-h fasting, insulin levels were lower in the treated WKY than in the untreated WKY (5.1 ± 1.1 vs. 2.1 ± 0.4 ng/ml, P<0.05). Plasma insulin responses were greater (P<0.05) in the untreated SHR than in the untreated WKY 5 and 10 min after glucose injection. Similarly, plasma insulin concentrations were higher in the SHR treated with CS-045 than in the treated WKY 0 min (P<0.05), 5 min (P<0.01) and 10 min (P<0.01) following glucose load. CS-045 treatment, however, did not affect plasma insulin response in SHR during the IVGTT.

Discussion

In the present study, we confirmed the results
of previous studies [14, 15], showing enhanced glucose tolerance and augmented insulin secretion in response to glucose in SHR. We recently found that glucose-induced insulin secretion from the isolated perfused pancreas was exaggerated in SHR compared to WKY (K. Tanigawa, submitted for publication). Whether insulin resistance plays a role in the development of hypertension in SHR is still a matter of controversy [4–6], but hyperinsulinemia is undoubtedly present in this animal. Hyperinsulinemia may reflect insulin resistance in euglycemic SHR. In addition, the present study revealed that WKY showed a much greater hyperglycemic response than SHR and queer insulin response. These findings led us to question whether WKY is the appropriate control for SHR. Pancreatic function and insulin sensitivity in peripheral tissues should therefore be compared in WKY and other normal Wistar rats.

Bleeding in our facility and aging increased blood pressure in WKY, but their blood pressure remained stable during the experimental period. Blood pressure in SHR was extremely high and further increased by aging. CS-045 partly prevented an age-related increase in blood pressure. Yoshioka et al. [16] recently demonstrated that CS-045 decreased both plasma insulin and blood pressure levels in obese Zucker rats, suggesting a potential link between insulin resistance and blood pressure regulation. In our study, however, plasma insulin concentrations were only partly decreased in SHR by CS-045 treatment. In addition, a decrease in blood pressure in SHR was not always associated with a reduction in plasma insulin concentrations. Since plasma insulin concentrations in Zucker rats were extremely high (36.4 ± 3.7 ng/ml) [14], CS-045 could be more effective in lowering the plasma insulin concentration than in SHR, resulting in a decrease in blood pressure. It is therefore possible to propose that hyperinsulinemia and/or insulin resistance is involved in the pathogenesis of hypertension in obese Zucker rats but not in SHR.

Zhang et al. [17] demonstrated that pioglitazone, one of the other thiazolidinedione derivative, attenuated the development of hypertension in both Dahl salt-sensitive rats and one-kidney, one clip rats. They suggested that the capacity of pioglitazone to reduce arterial pressure may not be directly related to its capacity to increase whole-body insulin stimulated glucose uptake. In the present study, CS-045 partly decreased blood pressure in SHR. Since aging increases insulin resistance [18], CS-045 might ameliorate enhanced insulin resistance by aging, resulting in a decrease in blood pressure. To clarify this, the effect of CS-045 on hypertension in young (8 weeks) SHR was examined for 8 weeks, and the results indeed revealed that CS-045 had no effect on plasma insulin or blood pressure levels in young SHR (data not shown). In addition, Katayama et al. [19] recently showed that CS-045 failed to decrease blood pressure in SHR despite ameliorating insulin sensitivity.

In conclusion, CS-045 partly decreased high blood pressure and plasma insulin concentrations in SHR, suggesting a very weak association between insulin resistance and hypertension in SHR.

References


