Inhibition of 11β-Hydroxysteroid Dehydrogenase Improves Glucose Metabolism in Insulin Resistant Otsuka Long-Evans Tokushima Fatty Rats

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(Received May 6, 2011; Accepted May 26, 2011)

Increased activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) has been implicated in the development of the metabolic syndromes by amplification of local glucocorticoid actions through regeneration of active glucocorticoid receptor. The present study was examined whether inhibition of 11β-HSD-1 by carbenoxolone (CBX), a non-selective 11β-HSD inhibitor, improved carbohydrate metabolism in insulin resistant Otsuka Long-Evans Tokushima Fatty (OLETF) rats or not. Rats received subcutaneous CBX, twice a day [50 mg/kg bodyweight (b.w.)] or vehicle for 2 weeks, and then were evaluated fasting blood glucose levels, glucose tolerance, serum fasting insulin levels, and blood lipid levels in the both groups. The fasting blood glucose and insulin were lower in CBX-treated OLETF rats at 2 weeks than those of compared to day 0 and vehicle-treated OLETF rats at 2 weeks. Blood glucose fluctuations of the CBX-treated OLETF rats were more normal than those of vehicle-treated ones during intraperitoneal glucose tolerance tests. Blood concentrations of cholesterol and free fatty acids in the CBX-treated OLETF rats were lesser than those of vehicle-treated ones. The area under time-concentration curve (AUC120 min) of blood glucose during the glucose tolerance test and of CBX-treated OLETF rats was significantly lower than that of the vehicle-treated ones, and insulinogenic index (ISI30 min) was significantly different between CBX- and vehicle-treated groups. These results suggested that inhibition of 11β-HSD-1 by CBX might be improved carbohydrate metabolism and lipid profile in the insulin-resistant OLETF rats.

Key words — 11β-hydroxysteroid dehydrogenase type 1, carbenoxolone, insulin resistance, type 2 diabetes, Otsuka Long-Evans Tokushima Fatty diabetic rat

INTRODUCTION

It is well known that glucocorticoids reduce insulin actions in insulin-sensitive tissues. Glucocorticoids stimulate hepatic gluconeogenesis, and reduce the insulin action which suppresses glucose production and utilization.1) The insulin antagonistic effects of glucocorticoids are well described in conditions of excessive glucocorticoids such as Cushing’s syndrome. Increased activity of glucocorticoids has been implicated in insulin resistance associated with type 2 diabetes mellitus and visceral obesity.2, 3) The effect of peripheral glucocorticoid is substantially determined through the interconversion of active cortisol and inactive cortisone under the influence of the 11β-hydroxysteroid dehydrogenase (11β-HSD).4) Two isoforms of 11β-HSD

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(type 1 and 2) have been characterized. While 11β-HSD-2, that is highly expressed in kidney, colon, sweat glands and placenta, functions as a unidirectional dehydrogenase which inactivates glucocorticoids,5, 6) 11β-HSD-1 functions as a bidirectional enzyme which predominantly acts as a reductase to catalyze the interconversion of active glucocorticoid hormones (corticosterone, cortisol) and inactive 11-keto metabolites (11-dehydrocorticosterone, cortisone).7) It is expressed in liver, adipose tissue and gonad,8, 9) and its local activity can be increased resulting in increased peripheral glucocorticoid actions without systemic elevation of glucocorticoids through regeneration of active glucocorticoids. Transgenic mice overexpressing 11β-HSD-1 demonstrate increased adipose levels of corticosterone and development of visceral obesity. These mice also showed marked insulin resistance, diabetes and hyperlipidemia.10) In contrast, the 11β-HSD-1 deficient mice showed attenuated activation of glucocorticoid-sensitive hepatic gluconeogenic enzymes in response to stress.11, 12) Recently, in human and obese Zucker rats, tissue specific alterations of 11β-HSD-1 activity have been implicated in the development of obesity and insulin resistance.13, 14) 11β-HSD-1 expression is increased in adipose tissues from obese individuals.15)

To investigate the role of peripheral glucocorticoid action in obesity-related type 2 diabetes, we examined the action of a peripheral glucocorticoid on glucose metabolism using a carbenoxolone (CBX), a non-selective 11β-HSD inhibitor in obese insulin-resistant Otsuka Long-Evans Tokushima Fatty (OLETF) rats that resemble the human type 2 diabetes characterized by late-onset and persistent hyperglycemia.

MATERIALS AND METHODS

Animals and CBX Treatment —— Male OLETF rats are characteristically doomed to begin to gain weight rapidly from 5 week-old, to begin insulin resistance at 8–9 week-old, and finally to develop obese type 2 diabetes. These OLETF rats were kindly supplied at 4 week-old from Otsuka Research Institute (OPC, Tokushima, Japan). They were housed in solid-bottom cages and were maintained in a room at 25°C with a 12–12 hr light-dark cycle. They were provided free access to a standard chow diet (Superfeed Co., Wonju, Korea) and water throughout the experiment. CBX (Sigma Co., St. Louis, MO, U.S.A.) treatment was commenced to animals at 15 week-old. OLETF rats (n = 6 per group) were injected subcutaneously with CBX [50 mg/kg bodyweight (b.w.)] in a total of 100 µl of saline, or vehicle (a matched volume of 0.9% sterile saline), twice daily (at 08:00–08:30 a.m. and at 07:00–07:30 p.m.) for 2 weeks. All the experimental procedures were certified by Institutional Animal Care and Use Committee of Hanyang University, Seoul, Korea.

Intraperitoneal (i.p.) Glucose Tolerance Tests —— At the 2 weeks of treatment, animals were fasted for overnight and underwent i.p. glucose tolerant tests at 09:00 a.m. using i.p. injection of glucose (2 g/kg b.w.). Blood was taken from the tail vein before (0), 30, 60 and 120 min after glucose loading. The blood glucose levels were measured using automated blood glucose analyzer (GLUCOSCOT-4310, Daiichi Co., Kyoto, Japan), and the area under the time-concentration curves (AUC; mg·min/ml) were calculated by the time-concentration plot during i.p. glucose tolerance tests for 120 min.

Biochemical Assay in Serum —— All animals were anesthetized by inhalation of ether and blood was collected by cardiac puncture. Serum was collected by centrifugation and stored at −80°C until subsequent biochemical assay for total triglyceride, cholesterol, and free fatty acids. Homeostasis model assessment of insulin resistance (HOMA-IR) and insulinogetic index at 30 min (ISI30min) were calculated during the i.p. glucose tolerant test, and serum insulin levels were measured using a rat insulin ELISA kit (Shibayagi Co., Shibukawa, Japan). The equations are as follows:

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\text{HOMA-IR} = \frac{\text{fasting insulin (µIU/ml)}}{\text{fasting glucose (mmol/l)}}/22.5
\]

\[
\text{and } \text{ISI30min} = 0.0077 \times \frac{\text{insulin30 min (pmol/l)}}{\text{blood glucose30 min (mmol/l)}}
\]

Statistical Analysis —— Data are presented as means ± S.E. Data were statistically analyzed by Student t-test and Mann-Whitney test using solar-powered satellite system (SPSS) 17.0. Differences between the groups were significantly considered at p < 0.05.

RESULTS

Body Weight and Food Intakes

There were no differences of daily food intakes
and body weight between CBX-treated OLETF rats and vehicle-treated OLETF rats for 2 weeks of treatment (data not shown).

**Fasting Serum Glucose, Insulin and Lipids**

Fasting blood glucose (FBS) levels were decreased after the 2 weeks of CBX treatment compared to pre-treatment levels (126.3 ± 10.0 vs. 109.5 ± 17.7 mg/dl, \( p < 0.05 \)), and FBS levels of the CBX-treated OLETF rats were lower than those of the vehicle-treated ones (109.5 ± 17.7 vs. 125.2 ± 9.2 mg/dl, \( p < 0.05 \), Fig. 1). Serum fasting insulin levels of CBX-treated OLETF rats were not changed when compared to those of vehicle-treated OLETF rats (data not shown), and HOMA-IR was not significantly different between CBX-treated OLETF rats and vehicle-treated ones (Fig. 2A). Two weeks of CBX treatment did not have any effects on serum triglyceride levels (Fig. 3A). However, it significantly decreased total cholesterol and free fatty acid levels in serum when compared to those of vehicle-treated OLETF rats (92.2 ± 11.7 vs. 115.1 ± 9.9 mg/dl, \( p < 0.01 \); 857.8 ± 235.1 vs. 1185.4 ± 142.1 µU/l, \( p < 0.05 \), respectively, shown in Fig. 3B and 3C).

**Intraperitoneal Glucose Tolerance Tests**

Blood glucose levels were monitored during i.p. glucose tolerance tests for 120 min after 2 weeks of CBX treatment, and the fluctuation of blood glucose levels of the CBX-treated OLETF rats were smaller than those of vehicle-treated ones (Fig. 4A). The AUC\(_{120\text{min}}\) of blood glucose during the glucose tolerance test for 120 min showed that AUC of CBX-
treated OLETF rats was significantly lower than that of the vehicle-treated ones (27792.5 ± 9127.7 vs. 40890.0 ± 5080.8 mg · min/ml, p < 0.05, Fig. 4B).

**DISCUSSION**

Glucocorticoids antagonize insulin actions on cellular glucose uptake and hepatic glucose input, acting mainly in the liver, adipose tissue and muscle via activation of intracellular glucocorticoid receptors. Increased action of glucocorticoid promotes expression of phosphoenol pyruvate carboxykinase, a rate-limiting enzyme in hepatic gluconeogenesis, and contributes to development of insulin resistance and type 2 diabetes. Therefore, limitation of glucocorticoid action can decrease phosphoenol pyruvate carboxykinase induction and hyperglycemia in diabetes mellitus. The mice with inactivated glucocorticoid receptor genes in hepatocytes showed hypoglycemia due to reduced expression of genes involved in gluconeogenesis. This suggests that agents reducing glucocorticoid action could be beneficial in control of hyperglycemia in diabetes.

Growing evidence shows that the pathogenic role of glucocorticoid would be rather dependent on regional glucocorticoid receptor activation by regenerating active glucocorticoid in the target tissues than systemic glucocorticoid levels. The activity of 11β-HSD-1 is an amplifier of intra-hepatic glucocorticoid action in vivo. Activation of local 11β-HSD-1 results in local hyperglucocorticoidism, and has been implicated in development of type 2 diabetes and obesity. In contrast, inhibition of glucocorticoid-induced transcription, due to a deficiency of local glucocorticoid generation in the 11β-HSD-1 knockout mice, induces attenuated gluconeogenic enzyme responses to stress and fasting. In hyperglycemic mice, administration of a selective 11β-HSD-1 inhibitor decreased the mRNAs of hepatic phosphoenol pyruvate carboxykinase, glucose-6-phosphatase, and the blood glucose concentrations. In the present study, OLETF rats, which provide an excellent model to study type 2 diabetes mellitus, were used. These rats were hyperphagic leading to accelerated rates of weight gain after 5 week-old, and eventually were developed hyperglycemic obesity with hyperinsulinemia and insulin resistance, as Kawano et al. reported. Administration of non-selective 11β-HSD-1 inhibitor, CBX for 2 weeks led to a decrease of fasting blood glucose in the OLETF rats (Fig. 1A), and it also significantly decreased FBS levels when compared to the results of vehicle administration in the present study (Fig. 1B). However, CBX did not induce the insulin sensitizing pattern of decreasing fasting insulin concentrations and HOMA-IRs, while Hermanowski-Vosatka et al. reported the decreased fasting insulin levels and HOMA-IRs by 11β-HSD-1 inhibitors. However, various results are presented through recent studies of CBX effect. In obese and hyperlipidemic mice, CBX administration had no effect on plasma glucose and insulin levels, however, it significantly decreased hyperinsulinemia in more severely
obese mice derived from heterozygous agouti (Ay/a) and homozygous low-density lipoprotein receptor (LDLR) −/− breeding pairs. In obese Zucker rats, CBX had no significant effects on plasma glucose, and it increased plasma insulin levels in the fasting state and at 30 min after glucose loading.\(^\text{23}\)

It is not clear how CBX improved glucose metabolism in this study. However, other studies presented possible explanations. 11β-HSD-1 mRNA was expressed in pancreatic islets isolated from pancreas in normal humans.\(^\text{24}\) In islets of Zucker Diabetic Fatty (ZDF, fa/fa) rats, both mRNA and enzymatic activity of 11β-HSD-1 increased in proportion to the hyperglycemia. Increased actions of local glucocorticoids in the islets result in impairment of glucose-stimulated insulin secretion and β-cell GLUT-2 expression.\(^\text{25}\) Therefore, it is possible that improvement of glucose tolerance with CBX might be partly attributed to increasing response of initial insulin secretion after glucose loading.

In conclusion, CBX is well known to alleviate glucocorticoids action in liver and adipose tissues which highly express 11β-HSD-1, even though the present study is lacking biochemical markers demonstrating improvement in adipocytokines or hepatic gluconeogenesis. And CBX reinforces the positive effect on the decreasing blood glucose levels in the OLETF rats, result in the overall improvement of glucose tolerance. These results suggested that CBX showed beneficial effects on the control of hyperglycemia in the insulin resistant OLETF rats, and support the notion that inhibition of 11β-HSD-1 might be an effective target in the control of type 2 diabetes.

Acknowledgements This work was supported by the research fund of Hanyang University, Seoul, Republic of Korea (HY-2005-S).

REFERENCES


