J Pharmacol Sci **115**, 329 – 335 (2011)

Journal of Pharmacological Sciences

Full Paper

Contributions of Hepatic Gluconeogenesis Suppression and Compensative Glycogenolysis on the Glucose-Lowering Effect of CS-917, a Fructose 1,6-Bisphosphatase Inhibitor, in Non-obese Type 2 Diabetes Goto-Kakizaki Rats

Taishi Yoshida[1,*], Akira Okuno[1], Kanako Takahashi[1], Junko Ogawa[1], Yuka Hagisawa[1], Shoichi Kanda[1], and Toshihiko Fujiwara[2]

[1]*Cardiovascular-Metabolics Research Laboratories,* [2]*R&D Planning Department, R&D Division, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

Received October 13, 2010; Accepted January 4, 2011

Abstract. Contributions of gluconeogenesis suppression in liver, kidney, and intestine as major gluconeogenic organs to the glucose-lowering effect of CS-917, a fructose 1,6-bisphosphatase inhibitor, was evaluated in overnight-fasted Goto-Kakizaki (GK) rats. CS-917 decreased plasma glucose by suppressing glucose release and lactate uptake from liver but not from kidney and intestine. These results suggest that hepatic gluconeogenesis suppression predominantly contributes to the glucose-lowering effect of CS-917 in GK rats. Moreover, the mechanism by which CS-917 decreased plasma glucose more in overnight-fasted GK rats than in non-fasted ones was investigated. Lactate uptake from liver was suppressed by 15 mg/kg of CS-917 in both states, but glucose release from liver and plasma glucose were decreased only in the overnight-fasted state. CS-917 at 30 mg/kg decreased hepatic glycogen content in both states and depleted it in the overnight-fasted state. In the non-fasted GK rats, co-administration of CS-917 with CP-91149, a glycogen phosphorylase inhibitor, suppressed hepatic glycogen reduction by CS-917 and decreased plasma glucose more than single administration of CS-917. These results suggest that gluconeogenesis suppression by CS-917 was counteracted by hepatic glycogenolysis especially in the non-fasted state and that combination therapy with CS-917 and CP-91149 is efficacious to decrease plasma glucose in GK rats.

Keywords: gluconeogenesis, glycogenolysis, fructose 1,6-bisphosphatase, glycogen phophorylase, diabetes mellitus

Introduction

Gluconeogenesis is one of the major pathways for endogenous glucose production, which results in glucose generation from the non-carbohydrate carbon substrates such as lactate, glycerol, and amino acids (1). Previous studies using magnetic resonance spectroscopy and/or the deuterated water method suggest that gluconeogenesis increases in type 2 diabetes patients (2). Thus, gluconeogenesis inhibitors are considered to be one of the potential targets for pharmacological intervention in type 2 diabetes (3).

CS-917 (MB06322) is an inhibitor of fructose 1,6-bisphosphatase (FBPase), one of the rate-limiting enzymes for gluconeogenesis, that catalyzes the conversion of D-fructose 1,6-bisphosphate to D-fructose 6-phosphate (4, 5). It has been reported that CS-917 decreased plasma glucose by suppressing gluconeogenesis and endogenous glucose production in various diabetic models such as Zucker diabetic fatty rats (5 – 7) and Goto-Kakizaki (GK) rats (8, 9). In a clinical study, CS-917 decreased the fasting plasma glucose level in type 2 diabetic patients (10).

The liver and kidney have been reported to be major organs for gluconeogenesis (11). In addition, recent studies have implicated that FBPase is expressed in the intes-

*Corresponding author. yoshida.taishi.bc@daiichisankyo.co.jp
Published online in J-STAGE on February 22, 2011 (in advance)
doi: 10.1254/jphs.10262FP

tine (12) and that the intestine is another gluconeogenic organ in addition to the liver and kidney (13). CS-917 has been reported to suppress gluconeogenesis in primary hepatocytes (5) and perfused rat kidney (6), but the contributions of the gluconeogenesis suppression by CS-917 in these organs to the glucose-lowering in vivo is not fully understood.

In order to investigate the primary organ in which CS-917 exerts its glucose-lowering effects, we examined the glucose and lactate balance in the major gluconeogenic organs such as the liver, kidney, and intestine in vivo in the overnight-fasted GK rats after a single oral administration of CS-917 by the simultaneous multiple blood sampling method (14, 15).

Our previous study revealed that CS-917 decreased plasma glucose without insulin secretion more effectively in the overnight-fasted state than in the non-fasted state in GK rats (9). To elucidate the mechanism by which CS-917 decreased plasma glucose more in the overnight-fasted state than in the non-fasted state, we compared glucose and lactate balance in the liver and the contributions of gluconeogenesis and glycogenolysis to glucose homeostasis after CS-917 administration in the overnight-fasted GK rats with those in the non-fasted GK rats.

Finally, the glucose-lowering effect of co-administrating CS-917 with CP-91149 (16), a glycogen phosphorylase inhibitor, was evaluated in GK rats. These studies allow us to further understand the influence of glycogenolysis on the glucose-lowering effect of CS-917 in GK rats in more detail.

Materials and Methods

Materials

CS-917 (L-alanine, *N,N′*-[[5-[2-amino-5-(2-methylpropyl)-4-thiazolyl]-2-furanyl]phosphinylidene]bis-, diethyl ester; $C_{21}H_{33}N_4O_6PS$, M.W. 500.56) and CP-91149, a glycogen phophorylase inhibitor, were synthesized at Daiichi Sankyo Co., Ltd. (Tokyo). For oral administration, CS-917 and/or CP-91149 were suspended in a vehicle composed of 1% carboxymethyl cellulose (Wako Pure Chemical Industries, Ltd., Osaka).

Animals

Male GK (GK/Jcl) rats were purchased at 6 weeks of age from CLEA Japan, Inc. (Tokyo) and acclimatized until they were over 27-weeks-old. Rat chow (FR-2; Funabashi Farm Co., Ltd., Funabashi) and water were given ad libitum. All the rats were housed under a 12-h lighting cycle in a controlled environment (room temperature: 23°C – 24°C, humidity: 45% – 69%). All animal care and experimental procedures were approved by the Daiichi Sankyo Animal Care and Use Committee.

Multiple blood sampling study in GK rats following a single-dose administration of CS-917

CS-917 (15 mg/kg) was orally administered to overnight-fasted and non-fasted GK rats (n = 9 – 10 per group). Non-fasted rats were deprived of chow just after administration. Blood samples were taken from the tail vein just before and 2.5 h after administration and centrifuged using a TH-1 rotor (Tomy Seiko Co., Ltd., Tokyo) at 13,000 × *g* for 5 min to obtain plasma.

At 3 h after administration, the rats were anesthetized by intravenous injection of 50 mg/kg pentobarbital sodium (Dainippon Sumitomo Pharma Co., Ltd., Osaka), and blood samples were simultaneously taken from the hepatic vein, portal vein, renal vein, and abdominal aorta in the overnight-fasted rats and from the hepatic vein and portal vein in the non-fasted rats as described in previous reports (14, 15). All the blood samples were centrifuged using a T3S6 rotor (Hitachi Koki Co., Ltd., Tokyo) at 2,000 × *g* for 15 min to obtain plasma.

Plasma glucose levels in each sample were measured using an enzymatic autoanalyzer (Glucoloader-GXT®; A&T Corp., Yokohama). Plasma lactate levels were determined by an enzymatic colorimetric assay using Determiner LA (Kyowa Medex Co., Ltd., Tokyo). The following equations were used for calculating the balance of glucose and lactate in each organ. The plus and minus values were regarded as release and uptake, respectively.

(hepatic release) = (plasma level in the hepatic vein) − (plasma level in the portal vein)

(intestinal release) = (plasma level in the portal vein) − (plasma level in the abdominal aorta)

(renal release) = (plasma level in the renal vein) − (plasma level in the abdominal aorta)

Comparisons of glucose-lowering effect, gluconeogenesis suppression, and glycogenolysis after single oral administration of CS-917 in GK rats between the overnight-fasted state and non-fasted state

Vehicle (expressed as control group), CS-917 (30 mg/kg), CP-91149 (300 mg/kg), or combination of CS-917 (30 mg/kg) with CP-91149 (300 mg/kg) was orally administered to overnight-fasted or non-fasted GK rats (n = 6 per group). Non-fasted rats were deprived of chow just after administration. Blood samples were taken from the tail vein just before and 4 h after administration and centrifuged using a TH-1 rotor (Tomy Seiko Co., Ltd., Tokyo) at 13,000 × *g* for 5 min to obtain plasma. Plasma glucose level in each sample was measured using an enzymatic autoanalyzer (Glucoloader-GXT®; A&T Corp., Yokohama).

For the purpose of evaluating gluconeogenesis in GK rats, [^{14}C]-labeled $NaHCO_3$ was injected into the rat tail

veins at the dosage of 800 μCi/kg at 4 h after the administration. The rats were sacrificed by decapitation 20 min after the injection for collection of truncated blood and hepatic tissue.

The specific activity of [^{14}C]-labeled glucose in truncated blood was measured using a liquid scintillation analyzer (TRI-CARB, model 2900TR; Perkin Elmer, Inc., Waltham, MA, USA), and gluconeogenesis rate in vivo in GK rats was determined as described (5). The obtained hepatic tissues were immediately snap-frozen in liquid nitrogen and stored at −80°C until analysis. Then the hepatic content of the glycogen was determined as described (17, 18).

Plasma glucose level and hepatic glycogen content just before the administration in the non-fasted and overnight-fasted GK rats were evaluated by using the rats without administration.

Statistical analyses

The results are expressed as the means ± standard error (S.E.M.). The statistical significance in the comparison with the control group was determined by Student's *t*-test. All the statistical analyses were performed using SAS System Release 8.2 (SAS Institute Inc., Cary, NC, USA), and a difference of $P < 0.05$ was considered to be significant.

Results

Changes of plasma glucose level after the administration of CS-917 in GK rats

Table 1 shows the changes of plasma glucose levels 2.5 h after the administration of CS-917 at dose of 15 mg/kg in overnight-fasted and non-fasted GK rats. CS-917 significantly decreased plasma glucose in the overnight-fasted state, whereas CS-917 tended to only decrease plasma glucose in the non-fasted state.

Effects of CS-917 on glucose and lactate uptake/release from the liver, kidney, and intestine in the overnight-fasted GK rats

The balance of glucose and lactate in the liver, kidney, and intestine after the administration of CS-917 in the overnight-fasted GK rats is shown in Fig. 1, a and b, respectively.

In the overnight-fasted control group, only the liver released glucose accompanied with lactate uptake. On the other hand, glucose was taken up and lactate was released in the kidney and the intestine.

CS-917 significantly suppressed glucose release with decreasing lactate uptake in the liver in the overnight-fasted GK rats. On the other hand, CS-917 decreased glucose uptake and lactate release in the kidney and the intestine.

Effects of CS-917 on glucose and lactate uptake/release from the liver in the non-fasted GK rats

The balance of glucose and lactate in the liver after the administration of CS-917 in non-fasted GK rats is shown in Fig. 2. CS-917 decreased lactate uptake without significant change in glucose release in the liver in the non-fasted GK rats.

Comparisons of glucose-lowering effect, gluconeogenesis suppression, and glycogenolysis after single oral administration of CS-917 in GK rats between the overnight-fasted state and non-fasted state

Plasma glucose levels at 4 h after administration of CS-917 and/or CP-91149 in non-fasted and overnight-fasted GK rats are shown in Fig. 3a. CS-917 significantly decreased plasma glucose both in the non-fasted state and overnight-fasted state, but more potently in the overnight-fasted state (46% reduction in plasma glucose in comparison with the overnight-fasted control group) than in the non-fasted state (26% of reduction in plasma glucose in comparison with the non-fasted control group). When CS-917 was co-administered with CP-91149, plasma glucose in the co-administered group was decreased more than in the groups administered CS-917 or

Table 1. Changes in the blood glucose levels after the administration of CS-917 in GK rats

Treatment	Overnight-fasted					Non-fasted				
	n	Pre (mg/dl)	Post (mg/dl)	Post − Pre (mg/dl)	*P*-value (vs. cont.)	n	Pre (mg/dl)	Post (mg/dl)	Post − Pre (mg/dl)	*P*-value (vs. cont.)
Control	9	119 ± 8	107 ± 4	−12 ± 8	–	9	313 ± 22	251 ± 13	−62 ± 17	–
CS-917	10	129 ± 5	57 ± 4	−72 ± 5	<0.0001	9	321 ± 20	321 ± 11	−95 ± 13	0.1446

Plasma glucose was measured just before (pre) and at 2.5 h after the administration (post) in GK rats. Difference of plasma glucose 2.5 h after the administration was calculated as "Post − Pre", and statistical significance in comparison with the control group was evaluated by Student's *t*-test. Data are expressed as the mean ± S.E.M.

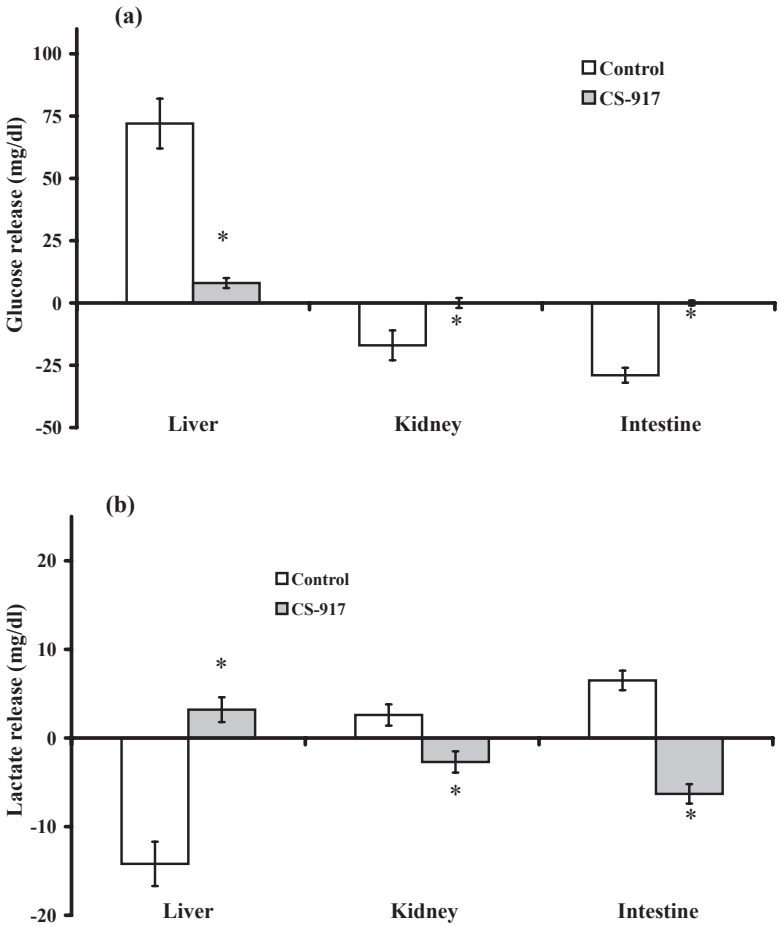

Fig. 1. Effects of CS-917 on glucose (a) and lactate (b) balance in the liver, kidney, and intestine in the overnight-fasted GK rats. Uptake/release of glucose and lactate in each tissue was calculated as described in Materials and Methods. Data are expressed as the mean ± S.E.M. (n = 9 – 10, $^{*}P < 0.05$, vs. control, Student's *t*-test; Control: open bar, CS-917: gray bar).

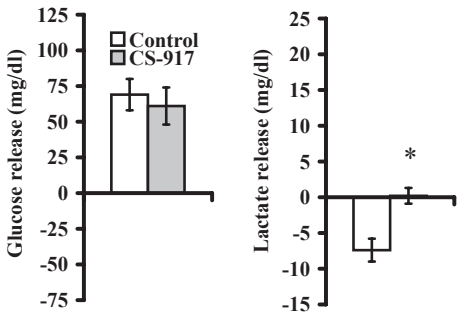

Fig. 2. Effects of CS-917 on glucose and lactate balance in the liver in non-fasted GK rats. Uptake/release of glucose and lactate in the liver was calculated as described in Materials and Methods. Data are expressed as mean ± S.E.M. (n = 9 – 10, $^{*}P < 0.05$, vs. control, Student's *t*-test; Control: open bar, CS-917: gray bar).

CP-91149 alone.

Gluconeogenesis rates at 4 h after the administration of CS-917 and/or CP-91149 are shown in Fig. 3b. Each gluconeogenesis rate was normalized to that in the non-fasted control group. Gluconeogenesis rate in the overnight-fasted group was significantly higher than that in the non-fasted group. CS-917 suppressed gluconeogenesis both in the non-fasted state and overnight-fasted state. CP-91149 administration had no significant effect on gluconeogenesis in both the control and CS-917 group in the non-fasted GK rats.

Hepatic glycogen contents at 4 h after the administration of CS-917 and/or CP-91149 are shown in Fig. 3c. CS-917 significantly decreased hepatic glycogen content both in the non-fasted state and overnight-fasted state. CP-91149 increased hepatic glycogen in the non-fasted state, but co-administration of CS-917 and CP-91149 caused no significant change in hepatic glycogen content in comparison with that in the non-fasted control group.

Discussion

Previous studies showed that CS-917 as an FBPase inhibitor suppressed gluconeogenesis from lactate in hepatocytes in vitro and decreased plasma glucose with the suppression of endogenous glucose production and gluconeogenesis in the whole body in vivo in type 2 diabetic rodents (5, 6, 8, 9). However, the quantitative contribution of hepatic gluconeogenesis suppression to the

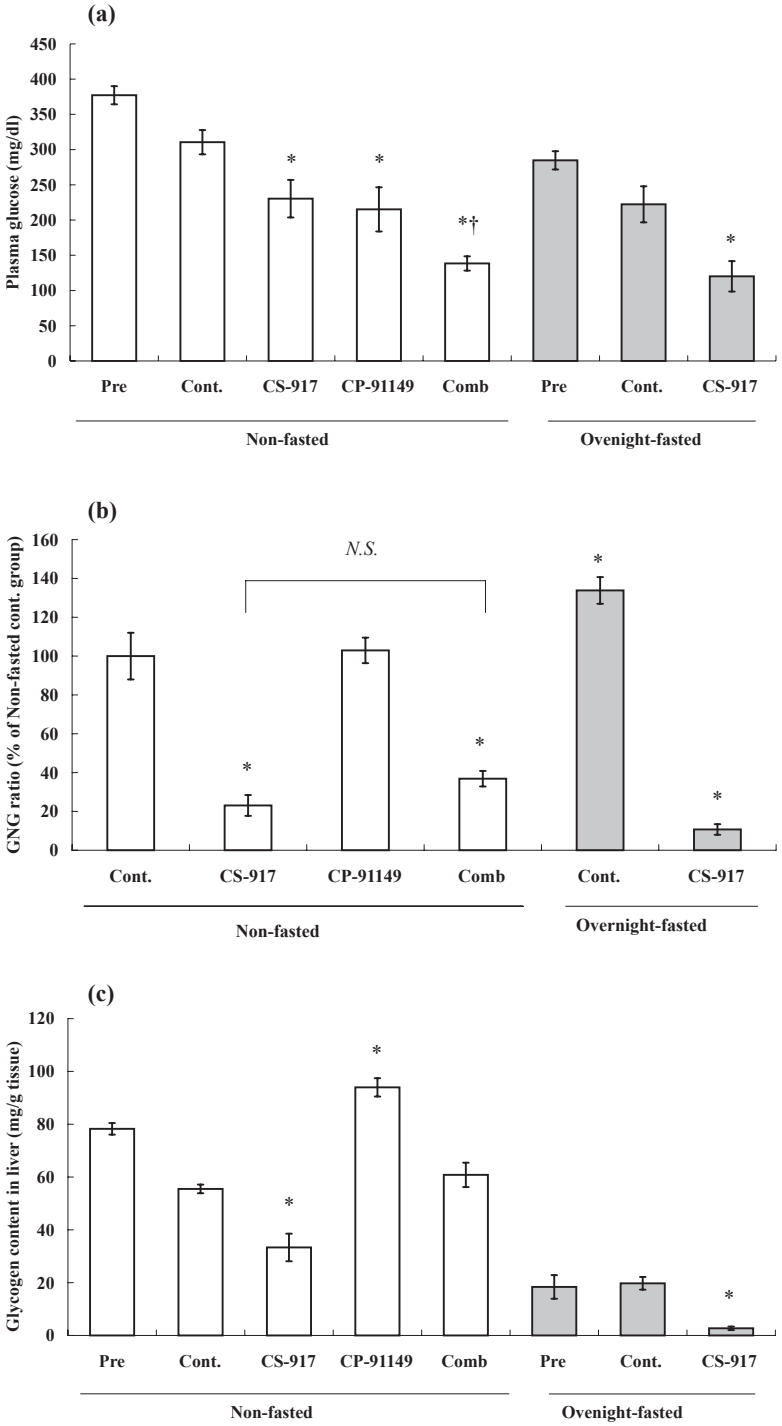

Fig. 3. Comparisons of glucose-lowering effect (a), gluconeogenesis suppression (b), and glycogenolysis (c) after single oral administration of CS-917 in GK rats between the non-fasted state (open bar) and the overnight-fasted state (gray bar). Plasma glucose level, gluconeogenesis ratio, and hepatic glycogen content at 4 h after the administration of vehicle (expressed as "Cont."), CS-917 (30 mg/kg), CP-91149 (300 mg/kg), or combination of CS-917 with CP-91149 (expressed as "Comb") were measured as described in Materials and Methods. Plasma glucose level and hepatic glycogen content just before the administration in non-fasted GK rats and overnight-fasted GK rats were evaluated by using the rats without administration (expressed as "Pre"). Data are expressed as the mean ± S.E.M. (n = 5 – 6, $*P < 0.05$, vs. control, $^{\dagger}P < 0.05$, vs. CS-917, Dunnett's test).

glucose-lowering effect of CS-917 has not yet been determined. In the present study, CS-917 markedly suppressed glucose release from the liver by suppressing lactate uptake as a gluconeogenic substrate while decreasing plasma glucose in the overnight-fasted GK rats as shown in Table 1 and Fig. 1. These findings suggest that the suppression of hepatic gluconeogenesis and glucose production predominantly contributed to the glucose reduction by CS-917 in the overnight-fasted GK rats.

The kidney is reported to possess sufficient gluconeogenic enzyme and to release glucose into the circulation as a result of gluconeogenesis, especially in a prolonged fasting state (19). Renal gluconeogenesis has been demonstrated to be higher in type 2 diabetic patients (20, 21), and the contribution of renal gluconeogenesis to plasma

glucose is almost equal to hepatic gluconeogenesis in obese patients in a prolonged fasting state (22). Recent ex vivo studies showed that renal gluconeogenesis was augmented 38% – 66% more in Zucker diabetic fatty rats than in the non-diabetic rats (23) and that CS-917 suppressed gluconeogenesis from lactate in a normal rat perifusion study (6). However, the present study showed that glucose was taken up by the kidney in the overnight-fasted diabetic GK rats, and that CS-917 did not decrease glucose release from the kidney in the overnight-fasted state, as described in Figs. 1 and 2. These results indicate that glucose uptake was superior to glucose production in the kidney in GK rats and that suppression of renal gluconeogenesis did not contribute to the glucose-lowering effect of CS-917 in GK rats.

Recent studies showed that the intestine also has sufficient gluconeogenic enzymes and has the ability to release glucose into the circulation as a result of gluconeogenesis (13). However, glucose was not released, but rather taken up by the intestine in the overnight-fasted GK rats. These findings suggested that intestinal gluconeogenesis did not contribute to glucose release from the intestine in GK rats. CS-917 did not affect the glucose balance in the intestine, as well as in the kidney. This result suggests that suppression of the intestinal gluconeogenesis did not contribute to the glucose-lowering effect of CS-917 in GK rats.

As shown in Table 1, CS-917 at dose of 15 mg/kg significantly decreased plasma glucose not in the non-fasted but in the overnight-fasted GK rats in accordance with our previous study (9). Gluconeogenesis rate in the overnight-fasted control group was higher than that in the non-fasted control group. These results confirm that the contribution of gluconeogenesis to glucose homeostasis is higher in the overnight-fasted state than in the non-fasted state as reported (24). CS-917 markedly suppressed the gluconeogenesis rate in the overnight-fasted state compared to in the non-fasted state as shown in Fig. 3b. These results suggest that enhanced gluconeogenesis and more potent suppression of gluconeogenesis by CS-917 in the overnight-fasted state were the mechanisms by which CS-917 decreased plasma glucose more in the overnight-fasted GK rats than in the non-fasted GK rats.

CS-917 at the dose of 15 mg/kg suppressed hepatic lactate uptake as an indicator of gluconeogenic suppression both in the non-fasted GK rats and the overnight-fasted ones, but hepatic glucose release was suppressed only in the overnight-fasted state as shown in Figs. 1 and 2. CS-917 concomitantly decreased hepatic glycogen both in the overnight-fasted and non-fasted state. These findings suggest that hepatic glycogenolysis counteracted the reduction of hepatic glucose release via gluconeogenesis suppression by CS-917. Hepatic glycogen content at 4 h after the administration of 30 mg/kg of CS-917 was completely depleted in the overnight-fasted state (2.7 ± 0.7 mg/g tissue) but still remained at a higher level in the non-fasted state (33.3 ± 5.2 mg/g tissue) as shown in Fig. 3c. These findings suggest that CS-917 decreased plasma glucose more in the overnight-fasted state than in the non-fasted state since further compensatory hepatic glycogenelysis could not operate in the overnight-fasted state.

The mechanism by which CS-917 induced compensative glycogenolysis was not fully understood. Recent hepatic perifusion study reported that both a glucose-6-phosphatase (G6Pase) inhibitor and a PEPCK inhibitor suppressed gluconeogenesis, but only a PEPCK inhibitor activated glycogenolysis with reduction of G6P content in liver (25). G6P was known to activate glycogen synthase and reduction of hepatic G6P content was also observed after administration of CS-917 in overnight-fasted GK rats (data not shown). These results suggest that attenuation of glycogen synthase by decrease in G6P might be one of the mechanisms by which CS-917 induced compensative glycogenolysis in GK rats.

Co-administration of CS-917 with CP-91149 decreased plasma glucose more than single administration of CS-917 not only in non-fasted GK rats as shown in Fig. 3a but also in overnight-fasted GK rats (data not shown). Gluconeogenesis suppression by CS-917 showed no significant change with or without CP-91149 treatment as shown in Fig. 3b, but compensative glycogenolysis induced by administration of CS-917 was cancelled by co-administration of CS-917 with CP-91149 as shown in Fig. 3c. These results suggested that co-suppression of gluconeogenesis and compensative glycogenolysis exert more potent glucose-lowering effect by suppressing hepatic glucose release more efficiently in GK rats.

In non-fasted GK rats, single administration of CP-91149 decreased plasma glucose with hepatic glycogen content higher than that in the pre administration as shown in Fig. 3: a and c. Shulman et al. reported that glycogen is directly synthesized from a gluconeogenesis substrate such as lactate and alanine as well as from glucose (26). The mechanism by which CP-91149 decreased plasma glucose with increasing hepatic glycogen content was not fully understood, but these results suggested that suppressing breakdown of glycogen synthesized from gluconeogenesis substrate might be one of the mechanisms.

In summary, hepatic gluconeogenesis suppression is a predominant contributor to the glucose-lowering effect of CS-917 in GK rats. CS-917 decreases plasma glucose more in the overnight-fasted GK rats than in the non-fasted ones because of higher gluconeogenesis and less

compensative glycogenolysis in the overnight-fasted state. CP-91149 improves the glucose-lowering effect of CS-917 by suppressing compensative glycogenolysis in the non-fasted GK rats. As such, both suppression of gluconeogenesis and glycogenolysis induced a potent glucose-lowering effect in the non-fasted GK rats. These results may have implications for CS-917 in terms of clinical usage for the treatment of type 2 diabetes.

Acknowledgment

We would like to thank Ms. Jenny Tarng of F.I.A. for her writing support.

References

1 Radziuk J, Pye S. Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. Diabetes Metab Res Rev. 2001;17:250–272.
2 Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Invest. 1992;90: 1323–1327.
3 Kurukulasuriya R, Link JT, Madar DJ, Pei Z, Rohde JJ, Richards SJ, et al. Prospects for pharmacologic inhibition of hepatic glucose production. Curr Med Chem. 2003;10:99–121.
4 Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver *in vivo*. Diabetes. 1999;48: 1198–1214.
5 Erion MD, van Poelje PD, Dang Q, Kasibhatla SR, Potter SC, Reddy MR, et al. MB06322 (CS-917): A potent and selective inhibitor of fructose 1,6-bisphosphatase for controlling gluconeogenesis in type 2 diabetes. Proc Natl Acad Sci U S A. 2005; 102:7970–7975.
6 van Poelje PD, Potter SC, Linemeyer DL, Erion MD. MB06322 (CS-917) lowers blood glucose in rodents by inhibiting both hepatic and renal gluconeogenesis. Diabetes. 2006;55 Suppl 1: A137.
7 Dang Q, Kasibhatla SR, Raja Reddy K, Jiang T, Rami Reddy M, Potter SC, et al. Discovery of potent and specific fructose-1,6-bisphopshatase inhibitors and a series of orally-bioavailable phosphoramidase-sensitive prodrugs for the treatment of type 2 diabetes. J Am Chem Soc. 2007;129:15491–15502.
8 Yoshida T, Okuno A, Izumi M, Takahashi K, Hagisawa Y, Ohsumi J, et al. CS-917, a fructose 1,6-bisphosphatase inhibitor, improves postprandial hyperglycemia after meal loading in non-obese type 2 diabetic Goto-Kakizaki rats. Eur J Pharmacol. 2008;601: 192–197.
9 Yoshida T, Okuno A, Tanaka J, Takahashi K, Nakashima R, Kanda S, et al. Metformin primarily decreases plasma glucose not by gluconeogenesis suppression but by activating glucose utilization in a non-obese type 2 diabetes Goto-Kakizaki rats. Eur J Pharmacol. 2009;623:141–147.
10 Bruce SR, Walker J, Feins K, Tao B, Triscari J. Initial safety, tolerability and glucose lowering of CS-917, a novel fructose 1,6-bisphosphatase (FBPase) inhibitor, in subjects with type 2 diabetes. Diabetelogia. 2006;49 Suppl 1:37.
11 Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, et al. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes. 1999;48:292–298.
12 Yánez AJ, Nualart F, Droppelmann C, Bertinat R, Brito M, Concha II, et al. Broad expression of fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase provide evidence for gluconeogenesis in human tissues other than liver and kidney. J Cell Physiol. 2003;197:189–197.
13 Mithieux G, Rajas F, Zitoun C. Glucose utilization is suppressed in the gut of insulin-resistant high fat-fed rats and is restored by metformin. Biochem Pharmacol. 2006;72:1257–1262.
14 Bailey CJ, Wilcock C, Day C. Effect of metformin on glucose metabolism in the splanchnic bed. Br J Pharmacol. 1992;105: 1009–1013.
15 Bailey CJ, Mynett KJ, Page T. Importance of the intestine as a site of metformin-stimulated glucose utilization. Br J Pharmacol. 1994;112:671–675.
16 Aiston S, Hampson L, Gómez-Foix AM, Guinovart JJ, Agius L. Hepatic glycogen synthesis is highly sensitive to phosphorylase activity: evidence from metabolic control analysis. J Biol Chem. 2001;276:30479–30486.
17 Hassid WZ, Abraham S: Chemical procedures for analysis of polysaccharide I. Determination of glycogen and starch. Determination of glycogen with the anthron reagents. In: Colowick SP, Kaplan NO, editors. Methods in enzymology. 3rd ed. New York: Academic Press; 1957. p. 35–36.
18 Kurosaki E, Momose K, Nakano R, Shimaya A, Suzuki T, Shibasaki M, et al. Hypoglycemic agent YM440 ameliorates the impaired hepatic glycogenolysis after glucose loading by increasing glycogen synthase activity in obese Zucker rats. Jpn J pharmacol. 2002;89:274–281.
19 Gerich JE, Woerle HJ, Meyer C, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. Diabetes Care. 2001;24:382–391.
20 Meyer C, Stumvoll M, Nadkarni V, Dostou JM, Mitrakou A, Gerich JE. Abnormal renal, hepatic, and muscle glucose metabolism in type 2 diabetes. J Clin Invest. 1998;102:619–624.
21 Meyer C, Woerle HJ, Dostou JM, Welle SL, Gerich JE. Abnormal renal, hepatic, and muscle glucose metabolism following glucose ingestion in type 2 diabetes. Am J Physiol Endocrinol Metab. 2004;287:E1049–E1056.
22 Owen O, Felig P, Morgan A, Wahren J, Cahill G. Liver and kidney metabolism during prolonged starvation. J Clin Invest. 1969; 48:574–583.
23 Eid A, Bodin S, Ferrier B, Delage H, Boghossian M, Martin M, et al. Intrinsic gluconeogenesis is enhance in renal tubules of Zucker diabetic fatty rats. J Am Soc Nephrol. 2006;17:398–405.
24 DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism. 1989;38:387–395.
25 Herling AW, Burger HJ, Schwab D, Hemmerle H, Below P, Schubert G. Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system. Am J Physiol. 1998;274: G1087–G1093.
26 Shulman GI, Rothman DL, Smith D, Johnson CM, Blair JB, Shulman RG, et al. Mechanism of liver glycogen repletion in vivo by nuclear magnetic resonance spectroscopy. J Clin Invest. 1985;76:1229–1236.