ELSEVIER

European Journal of Pharmacology 386 (1999) 75–82

ejp

www.elsevier.nl/locate/ejphar

Alterations of carbohydrate and lipid intermediary metabolism during inhibition of glucose-6-phosphatase in rats

Andreas W. Herling *, Hans-Joerg Burger, Gerrit Schubert, Horst Hemmerle, Hans-Ludwig Schaefer, Werner Kramer

Hoechst Marion Roussel Deutschland GmbH, H 821 Pharmacology, 65926 Frankfurt am Main, Germany

Received 5 August 1999; received in revised form 19 October 1999; accepted 22 October 1999

Abstract

S 4048 (1-[2-(4-Chloro-phenyl)-cyclopropylmethoxy]-3,4-dihydroxy-5-(3-imidazo[4,5-*b*]pyridin-1-yl-3-phenyl-acryloyloxy)-cyclohexanecarboxylic acid), a derivative of chlorogenic acid, specifically inhibits the glucose-6-phosphate translocating component T1 of the glucose-6-phosphatase system. Its pharmacological effect was studied on carbohydrate and lipid parameters in rats. In starved and fed rats, S 4048 caused a dose-dependent reduction of blood glucose levels with a corresponding increase in hepatic and renal glycogen and glucose-6-phosphate. The major quantitative route of carbon flux in the liver during S 4048-induced inhibition of the glucose-6-phosphatase activity seemed to be glycogenesis. Plasma free fatty acids were increased secondarily due to the S 4048-induced hypoglycemia. Hepatic triglycerides were increased possibly due to increased re-esterification of the readily available free fatty acids. Glucose-6-phosphate translocase inhibitors may be useful for experimentally studying aspects of type 1 glycogen storage disease in laboratory animals as well as for the therapeutic modulation of inappropriately high rates of hepatic glucose production in type 2 diabetes.

Keywords: Glucose production, hepatic; Glucose-6-phosphatase; Glucose, blood; Glycogenesis; Chlorogenic acid derivative

1. Introduction

Hepatic glucose production from both glucose producing pathways, gluconeogenesis and glycogenolysis, has an important impact on blood glucose levels and fuel homeostasis (Ashmore and Weber, 1959; Nordlie, 1974). The kidney is the only other organ capable of producing glucose. However, its contribution to whole body glucose production is still a matter of debate (Stumvoll et al., 1997; Ekberg et al., 1999). In both organs, the enzyme glucose-6-phosphatase (E.C. 3.1.3.9) in the endoplasmic reticulum catalyzes the terminal step of both glucose producing pathways (Nordlie, 1993).

According to the substrate-transport-model (Arion et al., 1975, 1980), hydrolysis of glucose-6-phosphate involves the coupled functions of three integral membrane proteins: (i) the catalytic unit situated with the catalytic site facing the lumen of the endoplasmic reticulum; (ii) a glucose-6-phosphate specific translocase T1, mediating penetration of the hexose-phosphate through the membrane, and (iii) a second translocase, denoted T2, that mediates efflux of inorganic phosphate.

During an overnight fast, hepatic glucose production protects the organism against hypoglycemia. However, inappropriately increased hepatic glucose production is a major symptom in type 2 diabetes and contributes significantly to fasting hyperglycemia (Reaven, 1997). Overexpression of glucose-6-phosphatase in normal rats with recombinant adenoviruses led to corresponding increases in enzyme protein and activity and was sufficient to perturb whole animal glucose and lipid homeostasis, possibly contributing to the development of metabolic abnormalities associated with diabetes (Trinh et al., 1998).

Absence of glucose-6-phosphatase activity in the liver and the kidney is the cause of glycogen storage disease type 1, an inherited disorder with abnormal hepatic storage of glycogen and fat, hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, and hyperlipidemia as clinical features (for review see Chen and Burchell, 1995). The

* Corresponding author. Tel.: +49-69-305-7565; fax: +49-69-305-81767.

E-mail address: andreas.herling@hmrag.com (A.W. Herling)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00748-7

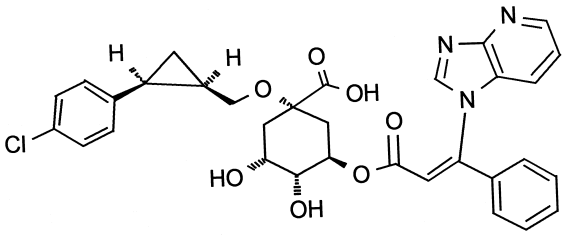

Fig. 1. Chemical structure of S 4048.

most frequent form of the disease is caused by mutations in the gene encoding the phosphohydrolase (Lei et al., 1993, 1995), whereas a less frequent but more severe form, termed glycogen storage disease type 1b, in which neutropenia and neutrophil dysfunction are additionally observed (Gitzelmann and Bosshard, 1993) is caused by defects in the glucose-6-phosphate translocase (T1) function (Narisawa et al., 1978; Lange et al., 1980). A gene encoding glucose-6-phosphate translocase T1 has recently been cloned (Gerin et al., 1997) and mutations in this gene were found in patients with glycogen storage disease type 1b (Veiga-da-Cunha et al., 1998). Interestingly, patients diagnosed as glycogen storage disease type 1c, with defects in the phosphate-translocase (T2), also had mutations in the glucose-6-phosphate translocase gene (Veiga-da-Cunha et al., 1998).

Recently, we described a novel class of compounds derived from the natural product chlorogenic acid (Arion et al., 1997; Hemmerle et al., 1997; Schindler et al., 1998) as specific and competitive inhibitors of the hepatic and renal glucose-6-phosphate translocase T1 (Arion et al., 1998b), but not of the phosphate translocase T2 (Arion et al., 1998a,b). In normal (non-diabetic) animal models the pharmacological profiles of the chlorogenic acid derivative S 3483 on hepatic glucose production, hepatic glycogen and glucose-6-phosphate contents as well as blood glucose profiles were fully consistent with its inhibitory effect on hepatic glucose-6-phosphatase activity (Herling et al., 1998). Parker et al. (1998) confirmed the pharmacological activity of S 4048, one of our chlorogenic acid derivatives, which they referred to as compound A, in diabetic animals.

Effects of chlorogenic acid derivatives on blood glucose levels and parameters of hepatic glucose metabolism have been demonstrated in vivo (Herling et al., 1998; Parker et al., 1998), however, a more detailed analysis of the metabolic alterations after pharmacological inhibition of T1 comprising also parameters of lipid metabolism as well as renal glucose metabolism has not been conducted. Here, we have used the chlorogenic acid analog S 4048 to investigate the alterations of carbohydrate and lipid metabolism in liver and kidney after inhibition of glucose-6-phosphatase function in non-diabetic rats and to compare the observed changes to the metabolic decompensation seen in human glycogen storage disease type 1.

2. Materials and methods

2.1. Test compound S 4048

S 4048 (1-[2-(4-Chloro-phenyl)-cyclopropylmethoxy]-3,4-dihydroxy-5-(3-imidazo[4,5-*b*]pyridin-1-yl-3-phenyl-acryloyloxy)-cyclohexanecarboxylic acid) (Fig. 1) was synthesized by the Chemistry Department at HOECHST MARION ROUSSEL Deutschland GmbH. S 4048 was dissolved in phosphate buffer, pH 7.4 containing 15% dimethylsulfoxide for intravenous administration.

2.2. Animals

Male Wistar rats (Hoe: WISKf (SPF 71), 230–250 g body weight) were used for the experiments. They were housed in groups of up to five per cage in a temperature-controlled room with a 12/12 h light/dark cycle. All animals had free access to water and to a standard pellet rat chow (Altromine 1320) unless otherwise indicated. Typical morning blood glucose levels were in the range of 4 to 5 mM in 24 h starved animals, and between 6 and 7 mM in fed rats. Liver glycogen content was below 30

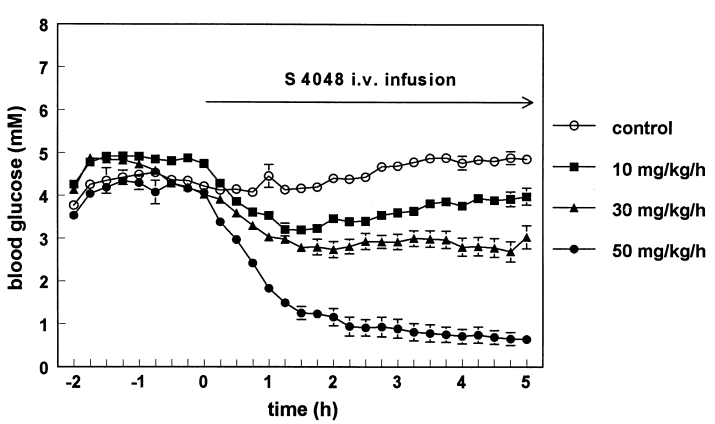

Fig. 2. Effect of S 4048 intravenous infusion on blood glucose levels in starved rats. Values are mean ± S.E.M.; $n = 6–14$ rats.

Table 1
Hepatic glycogen, glucose-6-phosphate, triglycerides and cholesterol as well as kidney glycogen and glucose-6-phosphate concentrations of in vivo freeze-clamped livers from rats at the end of the experiments shown in Figs. 2–4
Values are mean ± S.E.M.; n.d. = not determined.

Assay condition	Test compound	Dose (mg/kg/h i.v.)	*n*	Liver glycogen (μmol/g)	Liver glucose-6-phosphate (nmol/g)	Liver triglycerides (mg/g)	Liver cholesterol (mg/g)	Kidney glycogen (μmol/g)	Kidney glucose-6-phosphate (nmol/g)
Starved	control		14	16 ± 2	69 ± 4	2.0 ± 0.2	3.3 ± 0.2	2 ± 0.2	15 ± 0.5
	S 4048	10	6	30 ± 4[a]	88 ± 15	7.6 ± 1.5[a]	3.3 ± 0.2	13 ± 1[a]	17 ± 1
		30	10	92 ± 13[a]	91 ± 7[a]	7.7 ± 1.1[a]	3.4 ± 0.1	14 ± 2[a]	25 ± 3[a]
		50	6	252 ± 30[a]	821 ± 112[a]	9.5 ± 0.8[a]	2.7 ± 0.1	19 ± 2[a]	47 ± 2[a]
Fed	control		6	90 ± 18	140 ± 36	4.0 ± 0.3	2.6 ± 0.2	2 ± 0.2	16 ± 0.5
	S 4048	30	5	194 ± 10[a]	432 ± 90[a]	18.0 ± 4.1[a]	2.5 ± 0.1	11 ± 2[a]	36 ± 17
		50	4	390 ± 35[a]	430 ± 130[a]	n.d.	n.d.	n.d.	n.d.
Fed + glucagon	control		6	32 ± 8	515 ± 140	n.d.	n.d.	n.d.	n.d.
	S 4048	10	6	110 ± 20[a]	500 ± 80	n.d.	n.d.	n.d.	n.d.

[a] Indicates $P < 0.05$.

μmol/g, and between 200 and 300 μmol/g in starved and fed rats, respectively. The dramatic reduction of hepatic glycogen stores in rats starved for 24 h confirmed the well-known fact that in such animals the fasting blood glucose level is maintained predominantly by the process of gluconeogenesis.

2.3. Blood glucose profile in rats

Blood glucose levels were assayed in anaesthetized male Wistar rats as described previously (Herling et al., 1998). Briefly, rats were anaesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), tracheotomized, and one jugular vein per rat was cannulated for intravenous infusion. Anesthesia was maintained for up to 7 h by subcutaneous infusion of pentobarbital sodium (adjusted to the anaesthetic depth of the individual animal; about 24 mg/kg/h). Body temperature was monitored with a rectal probe thermometer, and temperature was maintained at 37°C by means of a heated surgical table. Blood samples for glucose analysis (10 μl) were obtained from the tip of the tail every 15 min, and for lactate analysis (20 μl) every 30 min. The rats were allowed to stabilize their blood glucose levels after surgery for up to 2 h. Then, S 4048 was infused intravenously (i.v.) for a period of 5 h at doses of 10, 30 and 50 mg/kg/h as indicated in the Section 3.

At the end of the experiment, the abdomen was opened, and a part of the liver and one kidney were freeze-clamped immediately. The frozen tissue was stored in liquid nitrogen for subsequent determinations of intrahepatic and intrarenal concentrations of glycogen, and glucose-6-phosphate. Total hepatic cholesterol and triglyceride contents were determined after homogenization of liver tissue and subsequent extraction with chloroform/methanol (Bligh and Deyer, 1959). The solvent was removed and the lipids redissolved in isopropyl alcohol (LiChrosolv E. Merck, Darmstadt, Germany). Total cholesterol and triglycerides were measured using Boehringer Mannheim enzymatic test kits (Cholesterol CHOD-PAP, Triglyceride GPOP-POP), with a Cobas Mira plus Analyser (Roche, Grenzach, SW). Blood was collected from the vena cava caudalis for determination of plasma concentrations of free fatty acids, triglycerides, cholesterol and uric acid. Standard enzymatic

Table 2
Blood parameters in rats at the end of the experiments shown in Figs. 2–4
Values are mean ± S.E.M.; n.d. = not determined.

Assay condition	Test compound	Dose (mg/kg/h i.v.)	*n*	Lactate (mM)	Free fatty acids (mM)	Triglycerides (mM)	Cholesterol (mM)	Uric acid (μM)
Starved	control		14	1.1 ± 0.1	0.6 ± 0.01	0.5 ± 0.04	1.2 ± 0.05	59 ± 3
	S 4048	10	6	1.9 ± 0.4	0.8 ± 0.05[a]	0.6 ± 0.06	1.4 ± 0.1	62 ± 5
		30	10	2.1 ± 0.4[a]	0.9 ± 0.06[a]	0.7 ± 0.05[a]	1.3 ± 0.1	90 ± 9[a]
		50	6	2.6 ± 0.4[a]	1.3 ± 0.2[a]	0.7 ± 0.1	1.1 ± 0.1	169 ± 40[a]
Fed	control		6	1.6 ± 0.1	0.4 ± 0.07	1.2 ± 0.2	1.6 ± 0.1	53 ± 5
	S 4048	30	5	1.7 ± 0.2	0.9 ± 0.1[a]	1.1 ± 0.2	1.6 ± 0.1	67 ± 7
		50	4	4.2 ± 0.9[a]	1.0 ± 0.1[a]	1.0 ± 0.2	n.d.	n.d.
Fed + glucagon	control		6	1.9 ± 0.4	0.5 ± 0.05	0.5 ± 0.1	n.d.	n.d.
	S 4048	10	6	1.9 ± 0.4	0.6 ± 0.1	1.0 ± 0.1[a]	n.d.	n.d.

[a] Indicates $P < 0.05$.

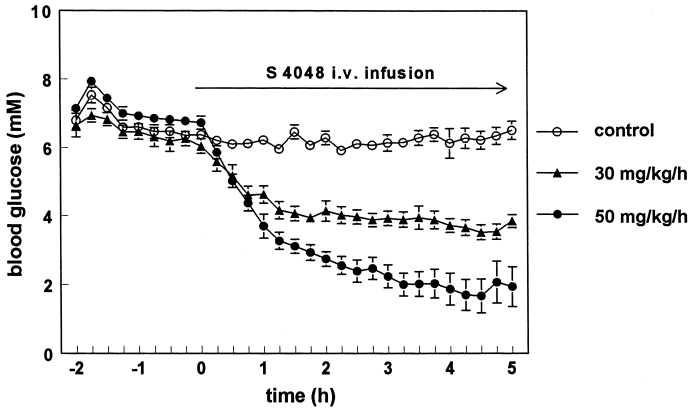

Fig. 3. Effect of S 4048 intravenous infusion on blood glucose levels in fed rats. Values are mean ± S.E.M.; $n = 4–6$ rats.

procedures were used to determine glucose, lactate, free fatty acids, triglyceride, cholesterol, glycogen, and glucose-6-phosphate (Bergmeyer, 1974). Insulin was measured by radioimmunoassay using a kit supplied by Biochem Immunosystems (Freiburg, Germany).

To study the effect of the test compound on the process of gluconeogenesis, rats were starved for 24 h since under these conditions euglycemia is maintained exclusively by gluconeogenesis due to the very low glycogen content in the liver. In normal fed rats, the pharmacological effect of S 4048 was studied assuming that under these conditions hepatic glucose output is matched by an autoregulatory mechanism between gluconeogenesis and glycogenolysis (Jenssen et al., 1990; Yki-Jarvinen, 1994). In contrast, in order to investigate the effect of the test compound solely on the process of glycogenolysis in rats that had also free access to food until the start of the experiment, glycogenolysis was induced by an i.v. bolus injection of glucagon at a dose of 0.5 mg/rat. It can be assumed that the hyperglycemia induced by the glucagon injection, and which lasted for about 90 to 120 min, was the result of the glucagon-induced breakdown of hepatic glycogen.

2.4. Statistics

Results are presented as the means and associated S.E.M. Statistical differences ($P < 0.05$) were assessed by Student's *t*-test; *n* indicates the number of animals.

3. Results

In starved rats, in which normoglycemia is maintained predominantly by the process of gluconeogenesis, S 4048 was infused i.v. at doses of 10, 30 and 50 mg/kg/h for 5 h. The blood glucose lowering effect of S 4048 was dose-dependent (Fig. 2) and nearly stabilized on dose-respective plateau values. Liver glycogen, glucose-6-phosphate, and triglycerides as well as renal glycogen and glucose-6-phosphate measured at the end of the study were dose-dependently and significantly increased; only the glucose-6-phosphate values in liver and kidney for the lowest

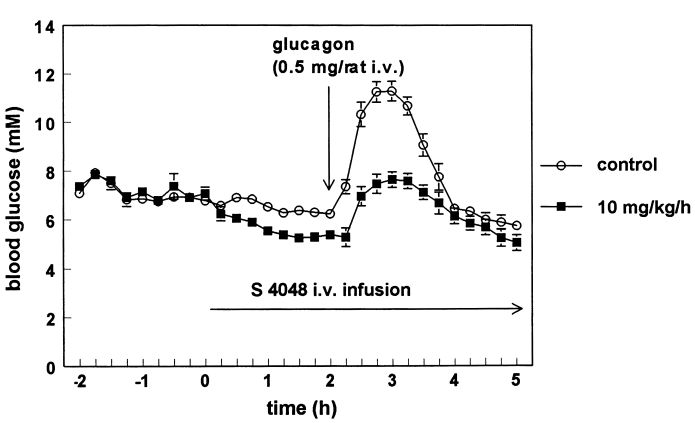

Fig. 4. Effect of S 4048 on blood glucose levels in fed rats given glucagon (0.5 mg/rat i.v.) 2 h after start of the S 4048 intravenous infusion. Values are mean ± S.E.M.; $n = 6$ rats.

Table 3
Plasma insulin levels in rats treated with S 4048
Values are mean ± S.E.M.

Assay condition	Test compound	Dose (mg/kg/h i.v.)	*n*	Insulin (μE/ml)
Starved	control		6	57.1 ± 6.6
	S 4048	50	4	28.6 ± 6.4[a]
Fed	control		4	105.5 ± 23.7
	S 4048	30	6	51.7 ± 5.8[a]

[a] Indicates $P < 0.05$.

dose were not significantly elevated (Table 1). In contrast to the liver triglycerides, the values for liver cholesterol were unchanged for all tested doses of S 4048 (Table 1). Blood levels for lactate, free fatty acids and uric acid at the end of the study were also dose-dependently increased, while only small changes, if any, were found in blood triglyceride and cholesterol levels (Table 2).

In normal fed rats, i.v. infusion of 30 and 50 mg/kg/h caused a dose-dependent reduction of blood glucose levels (Fig. 3) as well as a dose-dependent increase in liver glycogen, and glucose-6-phosphate (Table 1). There was also a significant increase in liver triglycerides and renal glycogen and glucose-6-phosphate values for the tested dose of 30 mg/kg/h, while liver cholesterol content was unchanged. Blood lactate and free fatty acids levels were also increased by S 4048, while plasma triglyceride, cholesterol and uric acid concentrations were not affected (Table 2).

In a second experiment with fed rats (Fig. 4), even the low dose of 10 mg/kg/h i.v. of S 4048 caused a slight lowering of the blood glucose level. In this study, glycogenolysis was provoked 2 h after the infusion of S 4048 by an i.v. injection of 0.5 mg glucagon per rat. Glucagon caused a marked, albeit transient hyperglycemic peak in control animals. In rats treated with S 4048, the hyperglycemic response was substantially reduced. Even at the end of this study, when blood glucose values of control and treated rats had returned to the normal range, liver glycogen content was still increased in the latter group, while glucose-6-phosphate was not different from control values (Table 1). Plasma free fatty acids were not changed, in contrast to plasma triglycerides which were significantly elevated (Table 2).

Effect of S 4048 on plasma insulin levels were determined at the end of separate experiments but otherwise identical to those in starved and fed rats described above. The hypoglycemia induced by S 4048 was paralleled by a decrease in plasma insulin levels (Table 3).

4. Discussion

In vivo, S 4048 reduced blood glucose levels dose-dependently (i) under conditions where normoglycemia in starved rats was predominantly maintained by gluconeogenesis, (ii) in normal fed rats where postprandial glucose levels were influenced by a matched process of gluconeogenesis and glycogenolysis, as well as (iii) in fed rats when the hyperglycemic peak after glucagon-induced glycogenolysis was reduced. At the end of these experiments, liver glycogen was increased in a dose-dependent manner in both normal fed and starved rats. Similar results were obtained for renal glycogen content. In 31-h starved rats (24 h prior the experiment plus the duration of the whole study) the infusion of 50 mg/kg/h of S 4048 for only 5 h caused the liver glycogen content to rise to 250 μmol/g, which is in the range of fed, control rats. Additionally, in starved and normal fed rats, hepatic and renal concentrations of glucose-6-phosphate were increased, showing dose-dependency, too. The increased glycogen could arise simply from a mass action effect of the increased levels of glucose-6-phosphate. However, direct activation of liver glycogen synthase by glucose-6-phosphate could also contribute to a redirection of the metabolic flux from glucose-6-phosphate hydrolysis towards the synthesis of glycogen (Villar-Palasi and Guinovart, 1997). From a theoretical point of view, with respect to the flux of carbon, it seems likely that the dynamics behind the increased glycogen content differ between starved and fed rats. In glucagon-injected fed rats, the high glycogen content at the end of the study likely is that which could not be mobilized by glucagon in treated rats due to inhibition of the hepatic glucose-6-phosphatase system. In contrast, in starved rats, the increased liver glycogen content is produced by neosynthesis using glucose-6-phosphate molecules that could not be hydrolyzed and released by the liver as glucose due to inhibition of glucose-6-phosphatase activity. The mild hyperlactatemia observed after inhibition of glucose-6-phosphatase for 5 h in normal rats is more likely the result of inhibited gluconeogenesis with subsequent accumulation of lactate due to the inhibition of the glucose-6-phosphatase system than of redirection of carbon flux into glycolysis.

The pharmacological profile of S 4048 resembled that observed earlier with another chlorogenic acid analogue, S 3483 (Herling et al., 1998) and is consistent with a specific inhibitory action on the glucose-6-phosphatase system in the liver of this more potent chlorogenic acid derivative. Similar results have been reported in mice and rats by Parker et al. (1998) using S 4048 referred to as compound A. Our study clearly demonstrates the dose-dependency of the pharmacological effect not only on blood glucose levels but also on liver glycogen and glucose-6-phosphate content. Furthermore, the study of renal parameters of carbohydrate metabolism showed dose-dependent effects of S 4048 on glycogen and glucose-6-phosphate consistent with an inhibition of carbon-flux through the glucose-6-phosphatase system also in the kidney.

Interestingly, in fed control rats with glucagon-induced glycogenolysis, glucose-6-phosphate was about three times

that of the fed control value listed in the previous study of Table 1 which is a necessary condition for the hyperglycemic response. The normalization of blood glucose in the absence of S 4048 most likely reflects accelerated removal of glucose by insulin-responsive tissues secondary to the increased insulin concentration presumed to be elicited both by the hyperglycemia and also directly by elevated glucagon. In this situation, the establishment of normoglycemia occurs in the presence of both, elevated glucose-6-phosphate levels that presumably cause an elevated rate of glucose production in the liver and a presumed elevated rate of uptake by the peripheral tissues. When S 4048 was present, hyperglycemia was prevented and therefore, a compensatory hyperinsulinemia did not occur, thus, normal removal of glucose occurred which was balanced by a ''normalized'' rate of glucose formation established by the competitive inhibition countering the elevated glucose-6-phosphate.

The blood glucose lowering effect during S 4048-treatment was not due to the concomitant hyperinsulinemia since the compound invariably caused a fall in the plasma insulin level that was commensurate with the degree of hypoglycemia (Table 3). Similar results have also been reported in mice by Parker et al. using S 4048 referred to as compound A (Parker et al., 1998). This conclusion is also supported by the results of an earlier in vitro study with the chlorogenic acid derivative S 3483 which appeared to have no effect on islet glucose-6-phosphatase activity (Khan et al., 1998).

For the first time, in addition to changes in carbohydrate metabolism, this study demonstrates a marked alteration of lipid metabolism during S 4048-induced hypoglycemic conditions. Plasma free fatty acids and hepatic triglycerides were significantly increased at the end of 5 h of exposure to S 4048. Plasma free fatty acids are predominantly an alternative source of fuel for the organism and their plasma levels are governed mainly by peripheral lipolysis in adipose tissues under the control of insulin. Thus, the increase in plasma free fatty acids in rats treated with S 4048 could easily be explained as secondary to the reduction of blood glucose and insulin levels. Accordingly, the hepatic triglycerides likely arise from re-esterification of the readily available free fatty acids, rather than lipogenesis, since the prevailing low insulin levels would not favor the latter. Reduced carbon flux through the glucose-6-phosphatase system caused by inhibition of hepatic glucose-6-phosphatase activity might result in increased levels of gluconeogenic intermediates also distal to glucose-6-phosphate including glycerol-phosphate that would support triglyceride biosynthesis.

Hyperuricemia, which is characteristic of glycogen storage disease type 1, was also seen in the present study, where a dose-dependent and significant increase in uric acid was induced by S 4048 (Table 2). Three possible causes of hyperuricaemia in glycogen storage disease type 1 are under debate: (i) reduced renal excretion of uric acid due to competition with lactate excretion, (ii) increased production due to increased availability of adenosine (accumulation of phosphate esters decreases intrahepatic phosphate and thus relieves the block on the hepatic AMP-deaminase), and (iii) increased production due to increased flux of carbon through the pentose-phosphate cycle and increased formation of phosphoribosyl-pyrophosphate and stimulation of nucleotide biosynthesis (Chen and Burchell, 1995). The mild hyperlactatemia seen in this study makes a competition between renal excretion of lactate and uric acid less likely. Also, increased production of uric acid from adenosine in combination with decreased ATP levels, as observed in glycogen storage disease type 1 patients (Greene et al., 1978), seems unlikely because ATP levels at the end of the 5-h treatment period were not reduced (data not shown). Because of the drastic increase in hepatic glucose-6-phosphate, it seems more likely that the hyperuricemia observed in the treated rats was caused by an increased carbon flux through the pentose-phosphate pathway with subsequent formation of phosphoribosyl-pyrophosphate and stimulated nucleotide and uric acid biosynthesis.

In summary, S 4048 reduced blood glucose in rats accompanied by increases in hepatic and renal glycogen, elevated blood lactate, caused hyperuricemia and a modest accumulation of hepatic triglyceride. Significantly, this metabolic profile is also seen, albeit in an exaggerated form, in patients suffering from glycogen storage disease type 1 (Burchell, 1990; Burchell and Waddell, 1993; Chen and Burchell, 1995). This genetic disorder is pathophysiologically characterized by the depression of glucose-6-phosphatase activity due to a defect in functionality of one of the integral components of the glucose-6-phosphatase system (Chen and Burchell, 1995). The corresponding animal model, represented by the glucose-6-phosphatase knockout mouse (Lei et al., 1996), showed similar symptoms with the exception that plasma lactate levels were not elevated suggesting adaptive mechanism in these mice different from the human condition. The common pathophysiological profile induced by S 4048 in vivo and seen in glycogen storage disease type 1 and with the exception of lactate also in the glucose-6-phosphatase knockout mouse lends credence to the conclusion that the glucose-6-phosphatase system is the primary pharmacological target of S 4048 in vivo.

An inappropriately high rate of hepatic glucose production occurs in type 2 diabetes (DeFronzo, 1988; Nielsen et al., 1990; Jeng et al., 1994). In light of the autoregulatory mechanism between glycogenolysis and gluconeogenesis (Jenssen et al., 1990; Yki-Jarvinen, 1994) and the observations presented and discussed above, inhibition of glucose-6-phosphatase appears to be the most efficient approach to pharmacologically modulate hepatic glucose production in type 2 diabetes. Furthermore, glucose-6-phosphate translocase inhibitors may be useful for experimentally studying aspects of carbohydrate and lipid metabolism comparable

to those observed in human glycogen storage disease type 1 in normal laboratory animals.

Acknowledgements

We would like to thank Dr. William J. Arion and Dr. J. Denis McGarry for their revisions of the manuscript and Dr. S. Efendic for his discussion of the project.

References

Arion, W.J., Wallin, B.K., Lange, A.J., Ballas, L.M., 1975. On the involvement of a glucose-6-phosphate transport system in the function of microsomal glucose-6-phosphatase. Mol. Cell Biochem. 6, 75–83.

Arion, W.J., Lange, A.J., Walls, H.E., Ballas, L.M., 1980. Evidence for the participation of independent translocation for phosphate and glucose-6-phosphate in the microsomal glucose-6-phosphatase system. Interactions of the system with orthophosphate, inorganic pyrophosphate, and carbamyl phosphate. J. Biol. Chem. 255, 10396–10406.

Arion, W.J., Canfield, W.K., Ramos, F.C., Schindler, P.W., Burger, H.J., Hemmerle, H., Schubert, G., Below, P., Herling, A.W., 1997. Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose-6-phosphatase. Arch. Biochem. Biophys. 339, 315–322.

Arion, W.J., Canfield, W.K., Callaway, E.S., Burger, H.J., Hemmerle, H., Schubert, G., Herling, A.W., Oekonomopulos, R., 1998a. Direct evidence for the involvement of two glucose-6-phosphate-binding sites in the glucose-6-phosphatase activity of intact liver microsomes. J. Biol. Chem. 273, 6223–6227.

Arion, W.J., Canfield, W.K., Ramos, F.C., Su, M.L., Burger, H.J., Hemmerle, H., Schubert, G., Below, P., Herling, A.W., 1998b. Chlorogenic acid analogue S 3483: a potent competitive inhibitor of the hepatic and renal glucose-6-phosphatase systems. Arch. Biochem. Biophys. 351, 279–285.

Ashmore, J., Weber, G., 1959. The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. Vitam. Horm. 17, 91–132.

Bergmeyer, H.U., 1974. Methoden der Enzymatischen Analyse. Verlag Chemie, Weinheim.

Bligh, E., Deyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911.

Burchell, A., 1990. Molecular pathology of glucose-6-phosphatase. FASEB J. 4, 2978–2988.

Burchell, A., Waddell, I.D., 1993. The molecular basis of the genetic deficiencies of five of the components of the glucose-6-phosphatase system: improved diagnosis. Eur. J. Pediatr. 152 (Suppl. 1), S18–S21.

Chen, Y.T., Burchell, A., 1995. Glycogen storage disease. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D. (Eds.), The Metabolic and Molecular Bases of Inherited Disease I McGraw-Hill, pp. 935–965.

DeFronzo, R.A., 1988. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37, 667–687.

Ekberg, K., Landau, B.R., Wajngot, A., Chandramouli, V., Efendic, S., Brunengraber, H., Wahren, J., 1999. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48, 292–298.

Gerin, I., Veiga-da-Cunha, M., Achouri, Y., Collet, J.F., van Schaftingen, E., 1997. Sequence of a putative glucose-6-phosphate translocase, mutated in glycogen storage disease type Ib. FEBS Letters 419, 235–238.

Gitzelmann, R., Bosshard, N.U., 1993. Defective neutrophil and monocyte functions in glycogen storage disease type Ib: a literature review. Eur. J. Pediatr. 152 (Suppl. 1), 33–38.

Greene, H.L., Wilson, F.A., Hefferan, P., Terry, A.B., Moran, J.R., Slonim, A.E., Claus, T.H., Burr, I.M., 1978. ATP depletion, a possible role in the pathogenesis of hyperuricaemia in glycogen storage disease type I. J. Clin. Invest. 62, 321–328.

Hemmerle, H., Burger, H.J., Below, P., Schubert, G., Rippel, R., Schindler, P.W., Paulus, E., Herling, A.W., 1997. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. J. Med. Chem. 40, 137–145.

Herling, A.W., Burger, H.J., Schwab, D., Hemmerle, H., Below, P., Schubert, G., 1998. Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system. Am. J. Physiol. 274, G1087–G1093, (Gastrointest. Liver Physiol. 37).

Jeng, C.Y., Sheu, W.H.H., Fuh, M.T., Chen, Y.D.I., Reaven, G.M., 1994. Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. Diabetes 43, 1440–1444.

Jenssen, T., Nurjhan, N., Consoli, A., Gerich, J.E., 1990. Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic autoregulation without a change in plasma glucose concentration. J. Clin. Invest. 86, 489–497.

Khan, A., Ling, Z.C., Herling, A.W., Landau, B.R., Efendic, S., 1998. Effects of 3-mercaptopicolinic acid and a derivative of chlorogenic acid (S-3483) on hepatic and islet glucose-6-phosphatase activity. Eur. J. Pharmacol. 349, 325–331.

Lange, A.J., Arion, W.J., Beaudet, A.L., 1980. Type Ib glycogen storage disease is caused by a defect in the glucose-6-phosphate translocase of the microsomal glucose-6-phosphatase system. J. Biol. Chem. 255, 8381–8384.

Lei, K.J., Shelly, L.L., Pan, C.J., Sidbury, J.B., Chou, J.Y., 1993. Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type Ia. Science 262, 580–583.

Lei, K.J., Pan, C.J., Liu, J.L., Shelly, L.L., Chou, J.Y., 1995. Structure–function analysis of human glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type Ia. J. Biol. Chem. 270, 11882–11886.

Lei, K.J., Chen, H., Pan, C.J., Ward, J.M., Mosinger, B., Lee, E.J., Westphal, H., Mansfield, B.C., Chou, J.Y., 1996. Glucose-6-phosphatase dependent substrate transport in the glycogen storage disease type-1a mouse. Nat. Genet. 13, 203–209.

Narisawa, K., Igarashi, Y., Otomo, H., Tada, K., 1978. A new variant of glycogen storage disease type I probably due to a defect in the glucose-6-phosphate transport system. Biochem. Biophys. Res. Commun. 83, 1360–1364.

Nielsen, H., Beck, O., Nielsen, H., 1990. On the determination of basal glucose production rate in patients with type 2 (non-insulin-dependent) diabetes mellitus using primed-continuous 3-3H-glucose infusion. Diabetologia 33, 603–610.

Nordlie, R.C., 1974. Metabolic regulation by multifunctional glucose-6-phosphatase. Curr. Top. Cell. Regul. 8, 33–117.

Nordlie, R.C., 1993. Recent advances in hepatic glucose-6-phosphatase regulation and function. Proc. Soc. Exp. Biol. Med. 203, 274–285.

Parker, J.C., Van Volkenburg, M.A., Levy, C.B., Martin, W.H., Burk, S.H., Kwon, Y., Giragossian, C., Gant, T.G., Carpino, P.A., McPherson, R.K., Vestergaard, P., Treadway, J.L., 1998. Plasma glucose levels are reduced in rats and mice treated with an inhibitor of glucose-6-phosphate translocase. Diabetes 47, 1630–1636.

Reaven, G.M., 1997. Pathophysiology of insulin resistance in human disease. Physiological Reviews 75, 473–486.

Schindler, P.W., Below, P., Hemmerle, H., Burger, H.J., Sreedhara Swamy, K.H., Arion, W.J., Efendic, S., Herling, A.W., 1998. Identification of two new inhibitors of the hepatic glucose-6-phosphatase system. Drug Development Research 44, 34–40.

Stumvoll, M., Meyer, C., Mitrakou, A., Nadkarni, V., Gerich, J.E., 1997. Renal glucose production and utilization: new aspects in humans. Diabetologia 40, 749–757.

Trinh, K.Y., O'Doherty, R.M., Anderson, P., Lange, A.J., Newgard,

C.B., 1998. Perturbation of fuel homeostasis caused by overexpression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. J. Biol. Chem. 273, 31615–31620.

Veiga-da-Cunha, M., Gerin, I., Chen, Y.T., de Barsy, T., de Lonlay, P., Dionisi-Vici, C., Fenske, C.D., Lee, P.J., Leonard, J.V., Maire, I., McConkie-Rosell, A., Schweitzer, S., Vikkula, M., Van Schaftingen, E., 1998. A gene on chromosome 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. Am. J. Hum. Genet. 63, 976–983.

Villar-Palasi, C., Guinovart, J.J., 1997. The role of glucose-6-phosphate in the control of glycogen synthase. FASEB J. 11, 544–558.

Yki-Jarvinen, H., 1994. The liver as a target for therapy in non-insulin dependent diabetes mellitus. Diabetes, Nutr. Metab. 7, 109–119.