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Review Article

Glucose-6-phosphate translocase as a target for the design of antidiabetic agents

Janice C. Parker

Pfizer Global Research & Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA.

CONTENTS

Introduction: current antidiabetic therapy

Diabetes mellitus is the most common of the endocrine diseases, afflicting more than 200 million people worldwide. Of these patients, 86-89% have type 2 diabetes (1). Type 2 diabetes is characterized by overproduction and underutilization of glucose, which together result in the fasting hyperglycemia that is the diagnostic criterion of diabetes.

Currently, type 2 diabetes is treated with a combination of diet and exercise, and then, if glycemic control is not achieved, with oral hypoglycemic agents followed by (or supplemented with) insulin, if necessary. Insulin remains the only effective therapy for type 1 diabetes. Antidiabetic agents currently marketed for the treatment of type 2 diabetes include: 1) insulin secretagogues such as the sulfonylureas (*e.g.*, glipizide, glyburide) and the newer, short-acting K^+ channel blockers (*e.g.*, nateglinide, repaglinide); 2) biguanides (*e.g.*, metformin); 3) α-glucosidase inhibitors (*e.g.*, acarbose); and 4) glitazones (*e.g.*, pioglitazone, rosiglitazone). α-Glucosidase inhibition targets absorption of dietary glucose from the gut (2, 3) and does not directly address the underlying metabolic defects that cause hyperglycemia. The insulin secretagogues stimulate insulin production by the pancreas and so promote insulin-sensitive glucose uptake and utilization (4, 5), but in some patients, they are ineffective due to coexisting and insurmountable insulin resistance. The glitazones (6-8) and biguanides (6, 9, 10) are insulin sensitizers; they act by alleviating insulin resistance and permitting endogenous levels of circulating insulin more effectively to facilitate uptake of glucose from the circulation and into the tissues. Metformin, which has multiple antidiabetic actions, has been reported to have some inhibitory effect on gluconeogenesis in type 2 diabetic patients (11, 12), but no currently available therapy directly targets hepatic glucose output, even though excessive hepatic glucose production is arguably a major cause of fasting hyperglycemia (13-15).

Hepatic glucose output

The two metabolic pathways by which the liver can produce glucose are gluconeogenesis and glycogenolysis (Fig. 1). When glycogenolysis is active, stored glycogen is broken down by the enzyme glycogen phosphorylase to produce glucose-1-phosphate, which is then converted to glucose-6-phosphate (G6P) before the phosphate is removed to liberate free glucose. Gluconeogenesis permits the *de novo* synthesis of glucose from 3-carbon precursors such as pyruvate and lactate. Estimates of the relative importance of the contributions of these two pathways to hepatic glucose production in the fed and the fasting state, and in normal health and in diabetes mellitus, vary (16-20). However, a single enzyme, glucose-6-phosphatase (D-glucose-6-phosphatase phosphohydrolase, E.C. 3.1.3.9; G6Pase), catalyzes the final step of both these pathways (Fig. 1), cleaving phosphate from G6P to liberate free glucose (21, 22). It follows that inhibition of G6Pase should reduce hepatic glucose production via both glycogenolysis and gluconeogenesis, regardless of their relative contributions to net hepatic glucose output and hence to glycemia. Indeed, since there are some reports that gluconeogenesis and glycogenolysis are coordinately regulated (23, 24), inhibition at a site that is capable of modulating both processes may be not just the most efficient, but possibly the only effective way of reducing total hepatic glucose production (25).

The glucose-6-phosphatase complex

G6Pase is a multicomponent enzyme system, represented schematically in Figure 2. The enzyme complex is

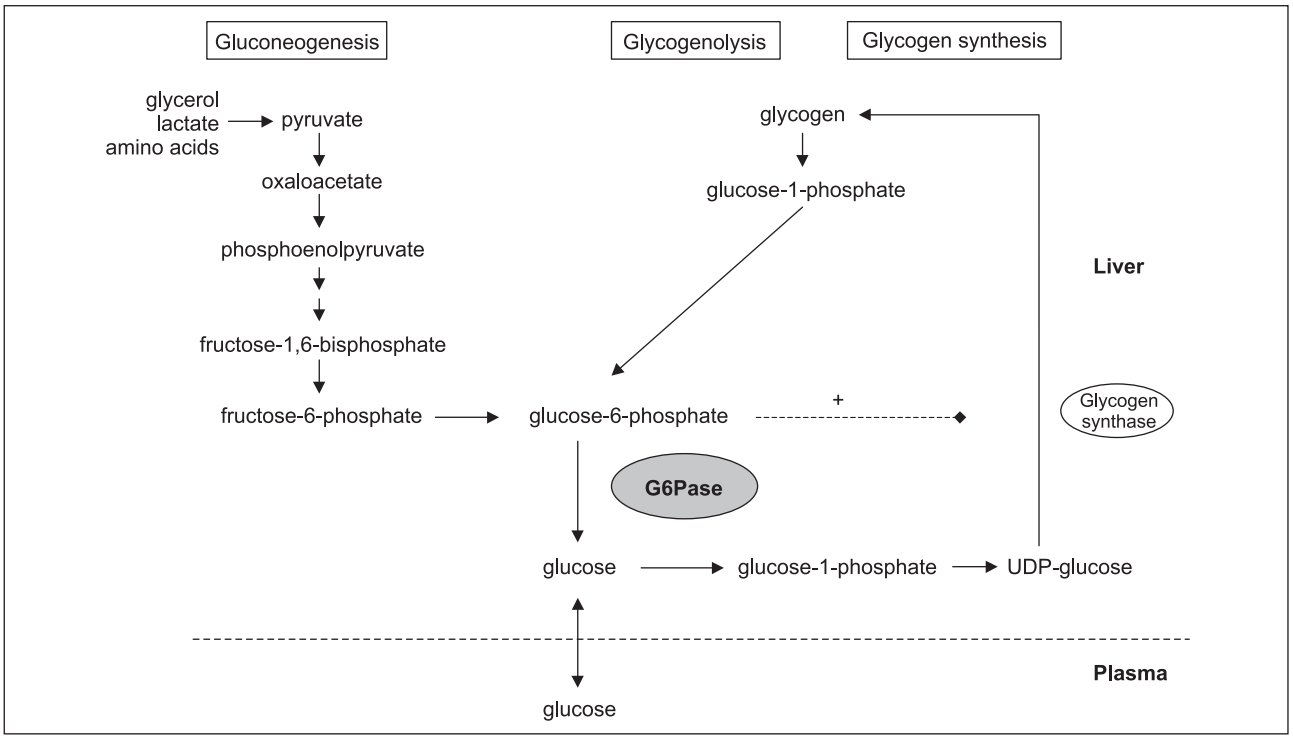


Fig. 1. Glucose-6-phosphatase catalyzes the final step of glucose formation in both the glycogenolytic and gluconeogenic pathways.

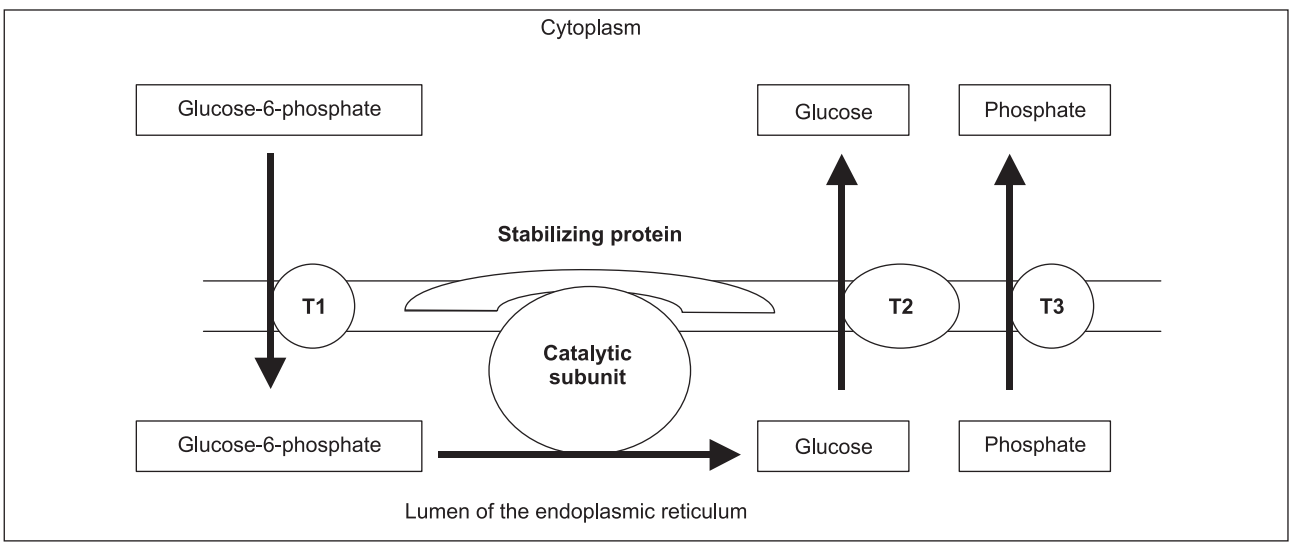
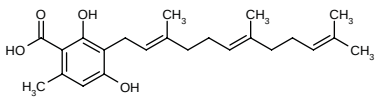
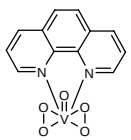
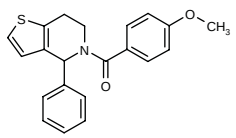


Fig. 2. Glucose-6-phosphatase is a multienzyme complex comprising transporters for glucose-6-phosphate (T1), glucose (T2) and phosphate (T3), in addition to the catalytic subunit and a stabilizing subunit.

an integral constituent of the membrane of the endoplasmic reticulum with its active catalytic site directed towards the lumen (26, 27). The complex comprises: (i) a transport protein, T1, which permits the entry of G6P into the ER; (ii) a catalytic subunit that cleaves the substrate G6P; (iii) two additional transporters, T2 and T3, that return the products, phosphate and glucose, respectively, to the cytoplasm; and (iv) a stabilizing protein, SP, that appears to function as a regulatory calcium binding protein (28). G6Pase is expressed primarily in the liver, but also to a lesser extent in the kidney (29), the pancreatic β-cell (30) and the mucosa of the small intestine (31). In the kidney, as in the liver, it is involved in gluconeogenesis, but it is also hypothesized to play a role in tubular reabsorption (32). In the β-cell, it appears to be involved in the mechanisms that coordinate glucose-dependent

Table I: Inhibitors of the glucose-6-phosphate catalytic subunit.

Compound	Structure	IC_{50} (μM)	Ref.
Ilicicolinic acid B		7.7	41
Oxodiperoxo(1,10-phenanthrolin)vanadate		0.96	36, 34
Tetrahydrothienylpyridine		0.61	42

triggering of insulin release (33). Its role in the intestinal mucosa is poorly understood, but it is hypothesized to be involved in glucose transport and absorption (32). Thus, the effects of introducing a G6Pase inhibitor *in vivo* may extend beyond the liver, and the possible consequences of inhibiting G6Pase activity in these other tissues must be taken into account.

Inhibitors of G6Pase

Inhibitors of the catalytic subunit

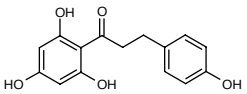
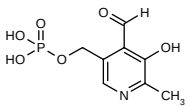
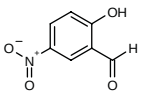
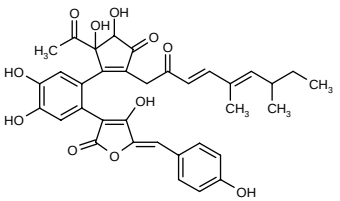
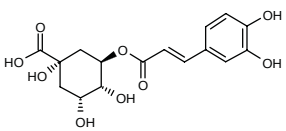
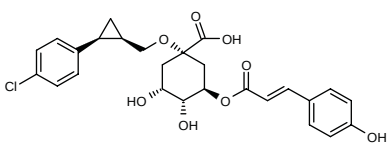
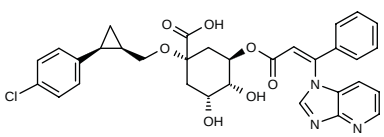
Peroxyvanadium compounds (Table I), which are inhibitors of the G6Pase catalytic subunit, have been shown to reduce glucagon-stimulated hepatic glucose output in the rat (34), although the *in vivo* effect may be attributable in part to the effects of these agents on other gluconeogenic enzymes such as fructose-2,6-bisphosphatase (35) and/or other phosphatases (36). Similarly, tungstate, which competes at the catalytic active site of G6Pase (37), lowers blood glucose in diabetic rats (38), although this appears to be attributable as much to improvements in pancreatic function (39) and/or insulin signal transduction (40) as to normalization of hepatic metabolism. This illustrates a potential liability of targeting the G6Pase catalytic subunit; agents that inhibit this enzyme are likely to inhibit other phosphatases, possibly with undesirable consequences. However, the nonspecific effects of tungstate and vanadate appear to be mostly insulin-mimetic and hence still consistent with a goal of glucose lowering. Ilicicolinic acid, a fungal product, has also been reported to inhibit G6Pase with micromolar potency (41), and the fact that this inhibition is still present in disrupted microsomes suggests that its site of action is the catalytic subunit. More recently, a new class of tetrahydrothienyl pyridines have been disclosed as G6Pase catalytic enzyme inhibitors (42). These are structurally distinct from the vanadate-based inhibitors, and more potent with IC_{50} values as low as 140 nM, but no data on their selectivity for G6Pase relative to other phosphatases have been published.

At present, specificity considerations suggest that the T1 translocase subunit may be the preferable target for pharmacological intervention since T1 exhibits a high degree of substrate specificity (43). No selective inhibitors of the T2 or T3 translocase have been identified to date, although agents with both T1 and T2 inhibitory activity have been described (44). Current knowledge of the function of the G6Pase enzyme complex (Fig. 2) suggests that inhibiting T2 or T3 would lead to an accumulation of glucose or phosphate, respectively, within the endoplasmic reticulum. This might serve to reduce G6Pase catalytic activity by a mass action effect; however, it would seem to raise the possibility of deleterious osmotic and/or metabolic effects on endoplasmic reticulum function.

Inhibitors of the T1 translocase

A number of inhibitors of the T1 translocase have been described (Table II), including phlorizin and its aglucone, phloretin (45), pyridoxal phosphate (46), taurocholate and sulfhydryl reagents (47), diazobenzene sulfonate (48), mercaptopicolinic acid (49) and *N*-bromoacetyl-ethanolamine phosphate (50). While these agents have proved useful tools for the study of G6Pase function, none has a high degree of specificity for T1. Recently, natural products have proved a major source of starting material for the design of more potent, specific T1 inhibitors. The active principle in *Bauhinia megalandra* leaves, used as a treatment for diabetes in Venezuelan traditional medicine, appears to be a T1 inhibitor (51), though its structure has yet to be resolved. The kodaistatins are T1 inhibitors isolated from extracts of *Aspergillus terreus* (52). Kodaistatin A and kodaistatin C,

Table II: Inhibitors of the T1 translocase.

Compound	Structure	IC_{50} (μM)	Ref.
Phloretin		340	41, 45
Pyridoxal phosphate		446	46, 41
2-Hydroxy-5-nitrobenzaldehyde		338	44, 41
Kodiastatin A		0.08	52
Chlorogenic acid		230	53
S-3438		0.2	54, 56
S-4048		0.002	53, 58

with IC_{50} values of 80 and 130 nM, respectively, for the inhibition of G6Pase activity in intact hepatic microsomes, are among the more potent of the T1 inhibitors identified thus far. Chlorogenic acid, the starting point for the design of most of the novel T1 inhibitors synthesized in recent years, is also a known plant constituent. Chlorogenic acid itself is a weak though selective inhibitor of T1 with an IC_{50} value of 230 μM (53), identified by routine screening conducted by researchers at Hoechst Marion Roussel (now Aventis Pharma). Another T1 inhibitor of comparable potency, hydroxynitrobenzaldehyde, which is structurally related to pyridoxal phosphate, was also identified, but further investigation revealed that this agent binds to both the T1 and T2 translocases (44). The Aventis researchers, by systematic modification of the molecule by combinatorial chemistry and rational drug design, were able to synthesize a series of specific T1 inhibitors with IC_{50} values in the nanomolar range (44, 54, 53). They correlated T1 inhibitory potency with chemical structure and, by use of computer-aided drug design, were able to generate a pharmacophore model that described the molecular interactions of the inhibitors with the T1 translocase. For optimal recognition, 2 hydrophobic interactions, 2 hydrogen bond acceptor functions and 1 negative ionizable group appeared to be necessary (55). The weak inhibitor chlorogenic acid was able to achieve only 3 of these 5 required interactions, while S-4048, a chlorogenic acid derivative designed to optimize all 5 interactions, had an IC_{50} value of 2 nM, 5 orders of magnitude more potent than that of the parent structure and the most potent T1 translocase inhibitor described so far.

Biological efficacy of T1 translocase inhibitors

The identification of potent, selective T1 inhibitors permitted proof-of-concept studies to examine the therapeutic efficacy of this mechanism. Herling *et al.* used the

chlorogenic acid derivative S-3438 to study the effects of T1 inhibition *in vitro* in isolated perfused rat livers and *in vivo* in normal rats (56). S-3438 inhibited hepatic glucose output from the isolated perfused livers of both fed and fasted rats, consistent with the proposal that an inhibitor of G6Pase should reduce glucose production via both the glycogenolytic and the gluconeogenic pathways. Intravenous infusion of S-3438 reduced blood glucose levels in fed rats and markedly reduced the excursion in blood glucose induced by glucagon stimulation of glycogen breakdown. Blood glucose concentrations were also reduced in starved rats infused with S-3438. Parker *et al.* working with a related chlorogenic acid derivative, Compound A (which is structurally identical to S-4048), also demonstrated glucose lowering in normal rats and mice and went on to document the antidiabetic effectiveness of this inhibitor in a rodent model of diabetes, the ob/ob mouse (57). Administered in conjunction with an oral glucose tolerance test, the inhibitor reduced glucose levels without increasing plasma insulin. In the treated obese mice the insulin levels were significantly lower, probably secondary to the treatment-induced reduction in plasma glucose concentration. A comparable fall in plasma insulin levels was reported for fed and fasted rats treated with S-4048 (58).

In these *in vivo* studies, the T1 inhibitors used were either infused i.v. (58, 56) or injected i.p. (57). This was necessary because systemic exposure after oral dosing was extremely low, probably due to poor intestinal absorption (57). The more potent of the chlorogenic acid derivatives also tend to be insoluble and lipophilic. Possibly the hydrophobic characteristics that are essential to an optimal interaction with the translocase also tend to militate against oral activity. To date, there have been no published reports of potent T1 inhibitors that are orally active.

Potential liabilities of T1 translocase inhibition

Since inhibition of G6Pase blocks both pathways of hepatic glucose production, there is theoretically the potential for inducing hypoglycemia by administering such an inhibitor. Compound A (S-4048) at single doses up to 100 mg/kg markedly reduced plasma glucose levels in animals fasted overnight to below 2 mM (< 36 mg/dl) (57). This effect was transient and euglycemia was restored within 3 h postdosing. Presumably counterregulatory mechanisms remained sufficiently intact to permit restoration of plasma glucose levels to a euglycemic range even when both pathways of hepatic glucose production were inhibited. Pharmacokinetic properties of the compound leading to a short duration of action may also have served to limit its hypoglycemic potential.

Another potential drawback of lowering plasma glucose levels by this mechanism is the possibility of elevating circulating lactate concentrations to unacceptable levels, and there is certainly evidence that inhibition of T1 translocase does result in increases in lactate levels both in the liver (57) and in the general circulation (58). In these studies the hyperlactemia observed was relatively mild and did not approach lactic acidosis.

In addition to a reduction in plasma glucose levels, inhibition of T1 translocase causes a variety of alterations in carbohydrate and lipid intermediary metabolism. Treatment of rodents with these agents has been reported to be associated with increased hepatic concentrations of G6P (58, 57), increased hepatic (58, 57) and renal (58) glycogen content, and increased triglycerides, cholesterol and uric acid (58). The reported increase in hepatic G6P levels is not surprising, given that the means of transporting G6P into the endoplasmic reticulum for breakdown to glucose is inhibited. The increase in glycogen accumulation could be a simple mass-action effect consequent upon the increased levels of G6P in the liver and presumably also in the kidney. However, it may also be that the synthesis of glycogen is promoted by a direct activation of glycogen synthase by G6P (59). The observed increases in cholesterol and triglycerides in S-4048-treated rats were interpreted to be due to reesterification of increased plasma free fatty acids, which occurred secondary to the reduction of blood glucose levels (58). However, there is preliminary evidence to suggest that S-4048 also directly increases *de novo* hepatic lipogenesis due to increased glycolytic flux and flux through hepatic acetyl-CoA precursor pools (60).

Defects in various components of the G6Pase complex have been implicated in a number of types of glycogen storage diseases (26). A deficiency in the T1 translocase manifests as glycogen storage disease type 1b (61). Patients present with hypoglycemia, lactic acidemia, hyperlipidemia and hyperuricemia as well as abnormal storage of glycogen. It appears that administration of T1 inhibitors results in a much milder and more benign but essentially comparable metabolic profile. Given these observations, it would certainly be appropriate to monitor plasma levels of lactate, cholesterol, triglycerides and uric acid as well as clinical markers of hepatic safety in any clinical studies using agents with this mechanism.

Conclusions

Published data support the hypothesis that a selective inhibitor of G6P T1 translocase could potentially be used as an antidiabetic agent. Animal studies with available inhibitors have demonstrated robust glucose lowering efficacy, with no notable acute side effects. The potential long-term efficacy and safety of these agents require further study. At the present time, it appears that the main obstacle to further preclinical or clinical evaluation is likely to be identification of an agent that combines the favorable *in vitro* potency of some of the chlorogenic acid derivatives with pharmacokinetic and biopharmaceutical characteristics consistent with oral activity, to support orally administered, multiple-dose studies.

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