Glycogen Phosphorylase-a is a Common Target for Anti-Diabetic Effect of Iridoid and Secoiridoid Glycosides

Hitesh B. Vaidya[1], Abeer A. Ahmed[1], Ramesh K. Goyal[2] and Sukhinder K. Cheema[1]

[1] Department of Biochemistry, Memorial University, St. John's, N, Canada. [2] Institute of Life Science, Ahmedabad University, Navrangpura, Ahmedabad, Gujarat, India.

Received, June 29, 2013; revised, August 6, 2013, accepted, September 6, 2013, Published September 9, 2013.

ABSTRACT – Purpose. Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, action or both. The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 B.C. One of the major problems with herbal drugs is that the active ingredients are not well defined. It is important to know the active components and their molecular interactions which will help to analyze their therapeutic efficacy and also to standardize the product. There are a number of medicinal plants known for their anti-diabetic effect that possess similarities in their active chemical components, e.g. iridoid and secoiridoid glycosides. **Methods**. In this study, we have compared the structure of various iridoid and secoiridoid glycosides to design a novel pharmacophore. We further developed a structure-activity relationship for the inhibition of glycogen phosphorylase-a. **Conclusion.** By using docking studies, we are proposing, for the first time, that inhibition of glycogen phosphorylase-a activity is a common target for iridoids and secoiridoids to elicit anti-diabetic effects.

INTRODUCTION

Type-2 diabetes mellitus accounts for 90-95% of all cases of diabetes. In 2010 the world prevalence of diabetes among adults (aged 20-79 years) was 6.4%, affecting 285 million adults; this number will increase to 7.7% and affecting 439 million adults by 2030 (1). Type-2 diabetes is characterized by defective insulin action leading to insulin resistance (2). Insulin resistance is associated not only with hyperinsulinemia and hyperglycemia, but also with other complications such as atherosclerosis, hypertension and abnormal lipid profile, collectively referred to as "Syndrome X" or Insulin Resistance Associated Disorders (3). A lipid profile consisting of a lower high-density lipoprotein (HDL)-cholesterol and a higher low-density lipoprotein (LDL)-cholesterol (atherogenic lipid profile) is a characteristic feature for type-2 diabetes. This atherogenic lipid profile is a result of increased hepatic very low-density lipoprotein (VLDL) synthesis, along with inhibition of VLDL clearance from circulation (4).

Currently, the treatment for type-2 diabetes relies mainly on a variety of approaches such as guanidine analogues (metformin), insulin sensitizers; sodium glucose transporter-2 (SGLT-2) inhibitors, glucagon like peptidase-1 (GLP-1) analogues and dipeptidyl peptidase-4 (DPP-IV) inhibitors, which are all intended to reduce hyperglycemia. However, these therapies have significant mechanism-based side effects, such as weight gain and atherosclerotic cardiovascular disease, as well as limited efficacy and tolerability (5). Therefore, therapeutic approaches that not only lower glucose level but also specifically address the diabetic dyslipidemia and atherosclerotic cardiovascular disease complications are needed (5).

In the last few years, there has been an exponential growth in the field of herbal medicine, which is gaining popularity in both developing and developed countries because of their natural origin and less side effects (6). Many conventional drugs have been derived from prototypic molecules in medicinal plants, such as the development of metformin, an efficacious oral glucose-lowering agent, was developed based on the use of *Galega officinalis* for the treatment of diabetes (7). Many

Corresponding Author: Dr. Sukhinder Kaur Cheema, Professor, Department of Biochemistry, Memorial University, St. John's, NL, Canada, Email: skaur@mun.ca

different plants have been used individually or in formulations for treatment of diabetes and its complications. One of the major problems with herbal formulation is that the active ingredients are not well defined. It is important to know the active components and their molecular interactions, which will help to analyze therapeutic efficacy.

A number of iridoid and secoiridoid glycosides have been shown to possess anti-diabetic and anti-hyperlipidemic effects in animal studies (8), suggesting involvement of a common mechanism of action. Thus, we compared the structural characteristics of iridoids and secoiridoids, and have proposed a new pharmacophore that may have a higher potency for anti-diabetic effect. Geniposide, an iridoid glycoside, was recently reported to have a hypoglycemic effect in streptozotocin induced diabetic mice (9), which was mediated by inhibition of hepatic glycogen phosphorylase-a (GPa's) and glucose-6-phosphatase (G6pase); two key enzymes in glycogenolysis. We therefore performed *in-silico* studies to confirm the proposed pharmacophore and its structure activity relationship using iridoid and secoiridoid glycosides as ligands and glycogen phosphorylase enzyme as the target protein. We are reporting, for the first time, a novel pharmacophore that may possess potent anti-diabetic activity via inhibiting *glycogen phosphorylase-a in vivo*.

Iridoid and secoiridoid glycosides

Iridoid and secoiridoid glycosides are the chemical class of compounds mainly reported in literature for their anti-hyperlipidemic, anti-diabetic and several other pharmacological effects (10). Iridoids are constituted of cyclopentanoid monoterpene derivatives and can be subdivided into four groups, iridoid glycosides, simple iridoids or non glycosidic iridoids, secoiridoids and bisiridoids (10). Their presence is a characteristic of angiosperms, in particular of the super order of sympetalae (11). The name "iridoid" was probably derived from the compounds such as an iridomyrcetin, iridolactone and iridoidal, isolated from a genus of ants known as *iridomyrex,* where these compounds are responsible for defensive secretion of ants (12). Sometimes they are referred to as "pseudoindicans" due to blue colorization developed upon hydrolysis. Iridoids are present in a number of folk medicinal plants and occur mainly, in dicotyledonous plant families like Apocynaceae, Scrophulariaceae, Diervillaceae, Lamiaceae, Loganiaceae and Rubiaceae (13). They were traditionally used as bitter tonics, sedatives, antipyretics, cough medicine, remedies for wounds, skin disorders and hypotensive agents (13). Intensive study of their bioactivity revealed that these compounds exhibit a wide range of bioactivities namely, cardiovascular benefits, hypoglycemic, hypolipidemic, anti-hepatotoxic, choleretic, anti-inflammatory, anti-spasmodic, anti-tumor, anti-viral, immunomodulator and purgative activities (14). Although iridoids were isolated in the latter part of the nineteenth century, the basic skeleton of these compounds was first proposed by Halpern and Schmid in 1958 based on their investigation on structure elucidation of plumieride (15).

Chemistry and biological activity of iridoids and secoiridoids

Iridoids are monoterpenoids based on a cyclopentane-[C]-pyran skeleton (Figure 1a**)**, which may consist of ten, nine, or rarely eight carbon atoms in which C11 is more frequently missing than C10 (11, 13, 14, 16, 17). Oxidative cleavage at 7,8-bond of the cyclopentane moiety affords the so called secoiridoids (Figure 1b) (11, 13, 14, 16, 17), while cleavage of pyran ring produced iridoids derivatives (Figure 1c) (17-20). The stereochemical configurations at C5 and C9 leads to *cis* fused rings, which is common to all iridoids containing the basic carbocyclic- or seco-skeleton in non-rearranged form. Iridoids and secoiridoids are the class of secondary metabolites of terrestrial and marine flora and fauna and are found in a wide variety of plants and also in some animals. In plant kingdom, these are derived from 9-hydroxy nerol by phosphorylation followed by cyclization, oxidation and glycosidation in several steps (21). Possibly, iridodial (Figure 1d) or 8-*epi*-iridodial (Figure 1e) is the predecessor of iridoids in many plant families (12, 22-24).

Secoiridoids are the metabolites of the iridoid glycosides and are monoterpenoids based on the 7, 8-secocyclopenta[c]-pyranoid skeleton (Figure 1f). In plants, they are possibly derived from iridoid, loganin by oxidative cleavage with redox enzyme and then undergo various secondary modifications of the basic skeleton namely, epoxidation, oxidation, hydroxylation and esterification of the generated hydroxyl groups, leading to a group of compounds, which constitute the secoiridoid class (8). We have identified a number of naturally occurring iridoid and secoiridoid glycosides that possess anti-diabetic and anti-hyperlipidemic effect.

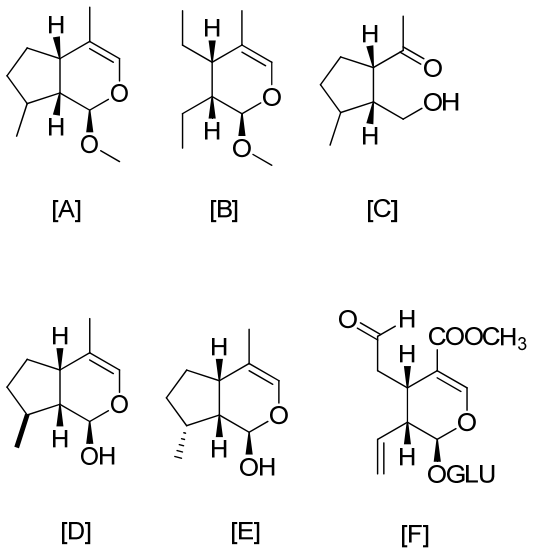

Figure 1. General chemical structure of iridoids and secoiridoids

The following sections deals with the chemical resemblance of iridoid and secoiridoid glycosides and their biological activity.

Swertiamarin

Swertiamarin is a secoiridoid glycoside (Figure 2) and is widely available in Gentianaceae family of plants such as *Ennicostema littorale* in India and China (25). We have previously reported that the aqueous, n-butanol and ethyl acetate extracts of *Enicostemma littorale* possess anti-diabetic and lipid lowering activity (26, 27). Later on, our group isolated swertiamarin, which was reported as one of the major components of n-butanol and ethyl acetate fractions (26), suggesting that swertiamarin may be the active compound for anti-diabetic and lipid lowering effect. Studies carried out in our laboratory with swertiamarin showed potent lipid lowering effect in both acute (Poloxamer-407 induced hyperlipidemia) (28) and chronic (high cholesterol diet induced) hyperlipidemic conditions (Figure 3) (29). We have also established anti-diabetic and anti-hyperlipidemic effects of swertiamarin in streptozotocin (STZ) induced type-1 and type-2 diabetes (30). Swertiamarin was also reported to have a number of pharmacological effects, namely hepatoprotective, anti-edematogenic, free radical scavenging activity and anti-spasticactivity (31).

Genipin

Genipin is classified under iridoids as its chemical structure is very similar to the general structure of iridoid glycosides (Figure 2**)**. Genipin is the molecule within the active fraction of the extract of *Gardenia jasminoides* Ellis fruits, which has been used over the years in traditional Chinese medicine to treat symptoms of type-2 diabetes (32, 33). It was reported that the acute inhibition of uncoupling protein-2 (UCP-2) by genipin reverses high glucose- and obesity-induced β-cell dysfunction (34). UCP-2 is a negative regulator of insulin secretion; it has been proposed that the increased expression of UCP-2 in β-cells could result in β-cell dysfunction and the development of type-2 diabetes (35). Genipin was reported to inhibit UCP-2 mediated proton leak, which eventually increased insulin secretion. In pancreatic β-islets, genipin increased mitochondrial membrane potential (by inhibiting proton leak) causing an increase in ATP levels, which eventually closes plasma membrane K_{ATP} channels, and stimulates insulin secretion in a UCP2-dependent manner (35).

Genipin is a naturally occurring cross-linking agent; two reactive hydroxyl groups of genipin form secondary structure by cross linking to each other (35). It is theoretically possible that the cross-linking activity of genipin could be required for inhibition of UCP-2; however it was later found that the cross-linking activity could produce adverse, nonspecific effects due to interaction with other proteins (35). To evaluate whether the cross-linking is responsible for the biological activity, a synthetic genipin-like compound, without the presence of cross linking free hydroxyl group, was synthesized and evaluated for UCP-2 activity.

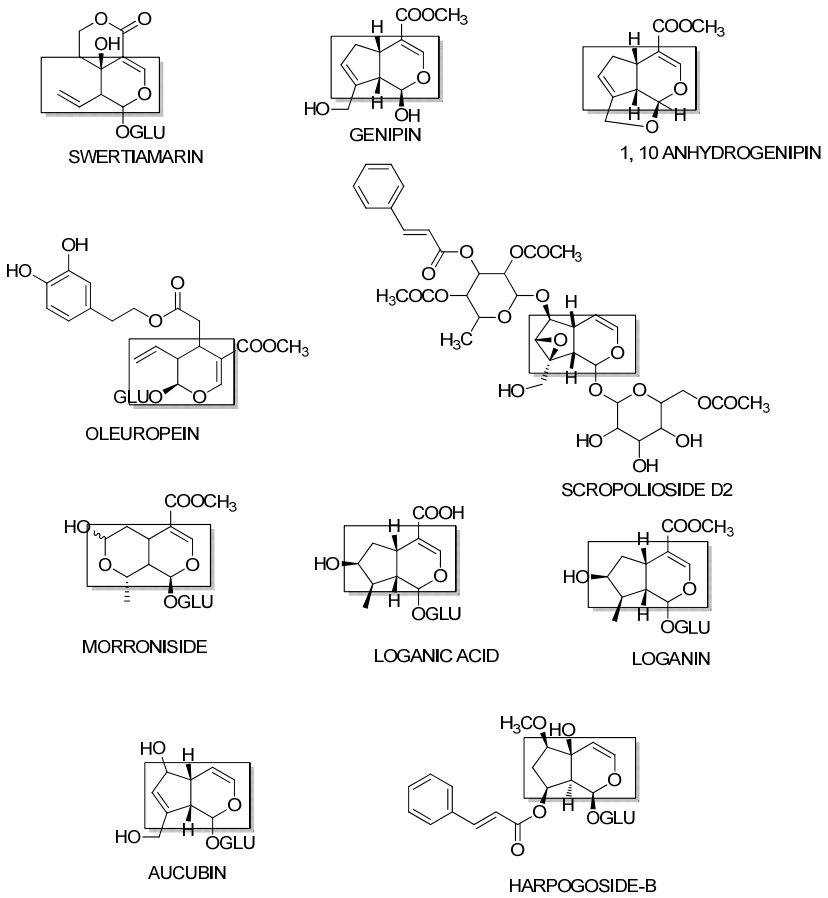

Figure 2. Iridoid and secoiridoid glycosides and their structural similarities

This compound was found to inhibit UCP2-mediated proton leak, closed K_{ATP} channels, and stimulated insulin secretion in a UCP2-dependent fashion (35). These findings suggest that the cross-linking activity of genipin is not required for its biological activity as a UCP inhibitor. Thus, the anti-diabetic effect of genipin appears to be independent of the cross-linking property and is likely due to the direct effect of genipin on insulin secretion, which may also involve other pathways (35).

Oleuropein

Oleuropein is a natural polyphenolic secoiridoid glycoside present in olive products, which scavenge free radicals and inhibit the chemical oxidation of LDL (36-38). Oleuropein is constituted of oxidized form of cyclopentane ring (open) and is also attached to the polyphenolic ring, thus it is categorized under polyphenolic secoiridoids (Figure 2). Diets rich in olive oil reduce tissue oxidative stress and enhance the glutathione antioxidant defense system in atherosclerotic rabbits (39), thus diets rich in polyphenols are currently recommended for the prevention of atherosclerosis (40, 41). Oleuropein has high antioxidant activity *in vitro*, comparable to a water soluble analog of tocopherol (42). Oleuropein also scavenges superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and hypochlorous acid derived radicals. The oxygen-derived free radicals contribute to the pathogenesis of atherosclerosis by oxidizing LDL and generating several species that react with oxygen in the vascular wall (43). Oleuropein was found to induce a significant hypoglycemic effect in alloxan-induced diabetic rats (44). The eventual mechanism responsible for the hypoglycemic activity of oleuropein and hydroxytyrosol may result from glucose-induced insulin release or increased peripheral uptake of glucose, and needs to be further investigated (45).

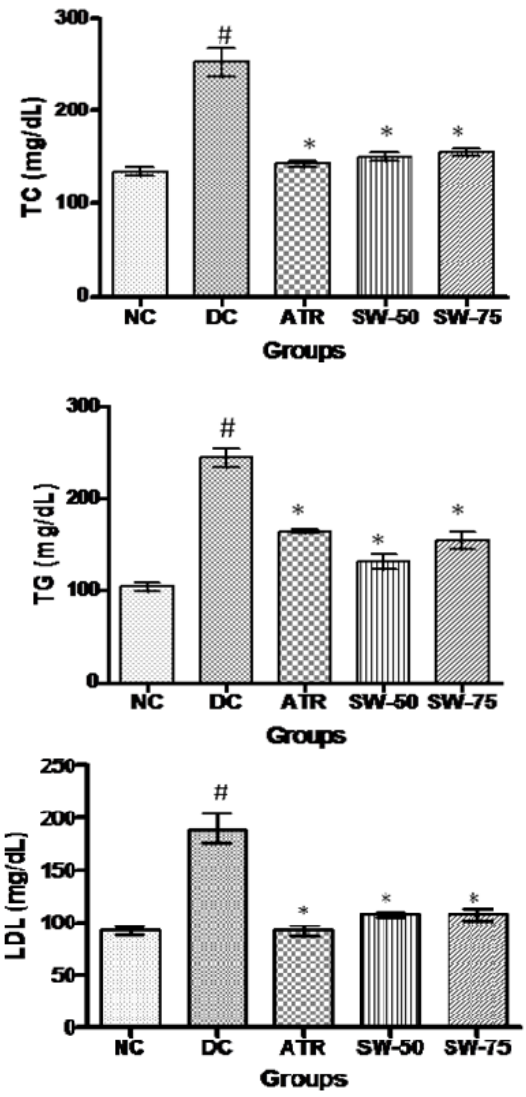

Figure 3. Effect of swertiamarin on total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL), in hypercholesterolemic rats fed a high cholesterol diet. Each bar represents mean ± SEM. (n= 6). NC = normal control, DC = disease control, ATR = atorvastatin treated (50mg/kg, p.o.), SW-50=swertiamarin treated (50mg/kg, p.o.), SW-75=swertiamarin treated (75mg/kg, p.o.). #Significantly different from normal control (P<0.001). *Significantly different from disease control (P<0.001).

Scropolioside-D2 and Harpagoside-B

Three known compounds namely, scropolioside-D2 (46), koelzioside (47), and 8-*O*-acetyl-harpagide are reported from the aerial parts of the plant *Scrophularia deserti* DEL (Scrophulariaceae), commonly known as *afinah*, *zetah*, *jar*, and *maseelah* in Saudi Arabia. The presence of cyclopentanepyran ring in scropolioside-D2 and harpagoside-B categorizes them under iridoid glycosides (Figure 2). *Scrophularia deserti* is known for antipyretic activity, a remedy for kidney diseases, cardiotonic, hypoglycemic, and diuretic in typhoid fever, galactorrhea, leuckorrhea, throat diseases, inflammation of mouth, lungs, large intestine, bladder, and heart. It also provides a remedy for tumors, abscesses, cancer of the lung, goiter, and aching bones (48). Furthermore, scropolioside-D2 and harpagoside-B have been reported to exhibit hypoglycemic activity in normal, fasting and alloxanized rats (49). The structure activity relationship (SAR) showed that scropolioside-D2 and harpagoside-B were the most active compounds to possess anti-diabetic and anti-inflammatory activity; however the mechanism/s of action is not yet established.

Loganic acid and Loganin

Loganic acid and loganin are the active molecules isolated from the genus *Cornus* (dogwood) belonging to the family Cornaceae, which consists of about 55 species, and is widely distributed in the northern hemisphere, Eastern Asia, and the Eastern and Northern parts of the United States (50). *Corni Fructus* belongs to the sub group Cornelian cherries containing loganin and loganic acid. Presence of cyclopentanepyran ring in the structure of loganin and loganic acid categorizes them under iridoid class. Hachimijiogan, morroniside and loganin are the major compounds (Figure 2) reported from *Corni Fructus* (*Cornus officinalis* SIEB. *Et* ZUCC), which helps in preventing diabetes related complications. It was previously discovered that *Corni Fructus* could ameliorate glucose-associated metabolic disorders (47). Their mechanisms were intimately related to the formation of advanced-glycation end products (AGEs) (51).

AGEs are a complex group of compounds formed via a non-enzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids. AGEs accumulate within various organs that are damaged in diabetes, with the accumulation rate of these AGEs accelerated by hyperglycemia (52). AGEs have been measured and reported to be linked to the sustained effects of prior glycemic control on the subsequent development of vascular complications. *Corni Fructus* has been used to improve liver and kidney functions. Iridoid total glycosides has the effect of preventing the over expression of transforming growth factor-b1 and matrixes in glomeruli using a diabetic model (53). *Corni Fructus* was also

reported for a better AGE clearance due to an improvement of renal function than aminoguanidine, the prototype AGE inhibitor. These findings suggest that *Corni Fructus* might be an effective remedy against diabetic AGE accumulation (51).

Aucubin

Aucubin is a common iridoid glycoside isolated from a number of plants namely, *Plantagoasiatica*, *Eucommiaulmoides*, the leaves of *Aucuba japonica* (54-56), and more recently from butterfly larva (57). Aucubin is a monoterpenoid based compound, and like all iridoids, has a cyclopentan-[C]-pyran skeleton. Iridoids can consist of ten, nine, or rarely eight carbons in which C11 is more frequently missing than C10; aucubin has 10 carbons with the C11 carbon missing (Figure 2). The stereochemical configurations at C5 and C9 lead to cis fused rings, which are common to all iridoids containing carbocyclic- or seco-skeleton in non-rearranged form as discussed earlier (58). Aucubin has been reported to have a number of pharmacological activities namely, hepatoprotective, anti-oxidant and anti-diabetic effects. Aucubin was also found to regenerate β-cells in streptozotocin induced diabetic rats (59), as well as inducing beneficial effects in diabetic complications such as neuropathy (60), however the exact mechanism of action is not known.

Proposed Common Mechanism of Action for Iridoid and Secoiridoid Glycosides

All of the iridoid and secoiridoid glycosides discussed above are known to possess anti-diabetic effects, however the proposed mechanism of action differ for each of the iridoid and secoiridoid glycosides. Since the chemical structure of all of the above compounds is similar, it is possible that there is a common biological pathway that is responsible for the anti-diabetic effect. Thus, we performed an *in-silico* docking study with a number of different insulin related targets, i.e. 2hr7 (insulin receptor "domains 1-3"), 1gag (insulin receptor kinase in complex with a bisubstrate inhibitor), and UCP-2, however none of these targets were specific for this class of compounds.

Wu et al., recently found that geniposide (iridoid glycoside) possesses anti-diabetic effect by inhibiting glycogen phosphorylase-a (GPa) and glucose-6-phosphatase (G6Pase) (8). GPa was considered as a possible target to treat diabetes as inhibition of GPa favors glycogen synthesis in both muscle and liver under high glucose conditions (61). GPa is an archetypical control enzyme and exists in two inter-convertible states: *A dephosphorylated form* (GPb, low activity and low substrate affinity) and *Ser 14 phosphorylated form* (GPa, high activity and high substrate affinity). Moreover GPa contains at least 6 potential regulatory sites: 1) the *Ser 14 P* recognition site, 2) the *AMP activatory site,* 3) the *glucose-6-P inhibitory allosteric* sites (GlcNAc), 4) the *catalytic site* that contains pyridoxal phosphate (PLP) at both sides, 5) the inhibitor site located at *12A* from the catalytic site, which binds *caffeine and related compounds*, 6) the *glycogen storage site,* and the *allosteric site* at the dimer interface of the protein (CP-403,700) (Figure 4).

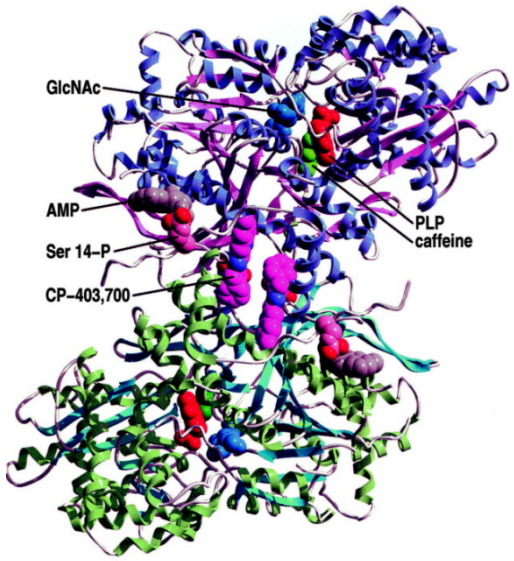

Figure 4. Binding sites for glycogen phosphorylase-a (Gpa). Adapted from protein data base (PDB), Adenosine monophosphate (AMP); pyridoxal phosphate (PLP); GlcNAc (glucose binding site); CP-403, 700 (glycogen storage site*)* (62).

Studies have shown that α-D-glucose is a weak inhibitor of GPa (k_i = 1.7 mM), and at the same time acts as a physiologic regulator of hepatic glycogen metabolism. Glucose binds to phosphorylase at the catalytic site and results in a conformational change that stabilizes the inactive T-state of the enzyme thereby promoting the action of protein phosphatase 1 and stimulating glycogen synthase.

We designed *in-silico* docking experiments to explore whether iridoid and secoiridoid glycosides would inhibit GPa, and whether there is any structure-activity relationship. First, a molecular modeling study was performed to investigate the possible binding conformations of iridoid and

secoiridoid glycosides to GPa that may suggest the mechanism of action. Docking study was then performed in two sets, first blind docking with GPa, followed by focused docking on the PLP binding site of the enzyme.

Docking calculations were carried out using Docking Server (63). The MMFF94 force field (64) was used for energy minimization of ligand molecules using Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on 1LWO-Transferase protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (65). Affinity (grid) maps of 20×20×20 A grid points and 0.375 A spacing were generated using the Autogrid program (65). AutoDock parameter set- and distance- dependent dielectric functions were used in the calculation of the van-der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (66). Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2500000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 A, and quaternion and torsion steps of 5 were applied. The activity of the molecules is considered to be due to the stabilization attained by the ligand-receptor complex, which is directly proportional to the magnitude of the interaction energy between them. These interaction energies principally scored in terms of hydrophobic, hydrogen bonding, polar and unfavorable steric contacts that have been calculated.

Blind docking of iridoid and secoiridoid glycosides on GPa showed no cluster to possess significantly higher frequency or lower energy compared to the other clusters. Thus, blind docking was not able to clearly define a possible binding site. We further looked into the molecular structure of the enzyme GPa to identify potential binding sites. The cluster possessing the highest interaction surface between the docked ligand and the protein was located at the PLP binding site. Thus, focused docking calculations were carried out at the PLP binding site of the protein.

All iridoid and secoiridoid glycosides were proven to bind at the PLP binding site of GPa with a reasonable affinity (-3.82 to -9.45 Kcal/mol, Table 1), except scropolioside D2 that did not show GPa inhibition (+36.97 Kcal/mol). Morroniside, with its docking energy of -6.69 Kcal/mol, suggests that the binding of iridoid and secoiridoid glycosides to GPa was glucose independent. Figure 5 shows the binding of morroniside (green color) with PLP binding site, which revealed a better binding affinity for morroniside (-6.69 Kcal/mol) compared to α-D-glucose (-5.00 Kcal/mol). Also, this binding indicated a lack of overlap between glucose (pink color) and morroniside, suggesting that inhibition of GPa by morroniside does not depend on the glucose moiety. Moreover, morroniside has shown a number of hydrogen bond interactions with many amino acids in the binding pocket; this interaction is also stabilized by a number of hydrophobic interactions with a number of amino acid residues at the binding site namely, Tyr90, ILE165, VAL567, TYR648.

Table 1. Docking energies of iridoids and secoiridoids at the pyridoxal phosphate (PLP) binding site of glycogen phosphorylase-a

Compound	Docking energy (kcal/mol)
Anhydrogenipin	-6.3
Aucubin	-7.38
Genipin	-5.69
Glucose	-5.00
Harpogoside B	-9.45
Loganic acid	-7.78
Loganin	-6.46
Morroniside	-6.69
Oleuropein	-3.82
Swertiamarin	-7.01
Scropolioside D2	+36.97

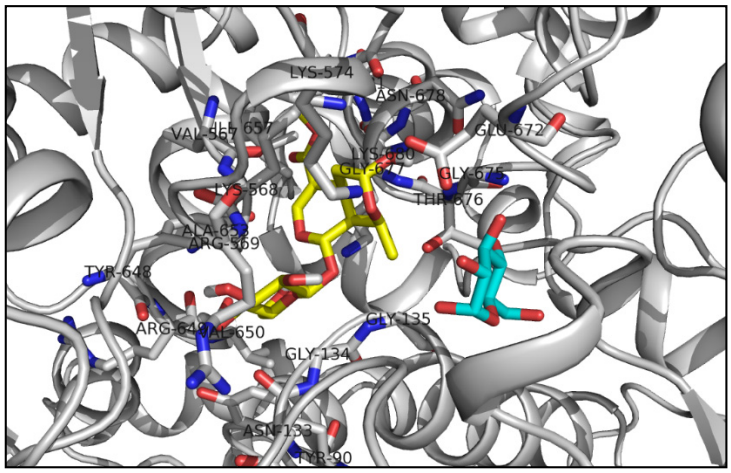

Figure 5. Docking poses of Morroniside (yellow) and Glucose (blue) docked into the pyridoxal phosphate (PLP) binding site (PDB code 1LWO) of glycogen phosphorylase-a (Gpa)

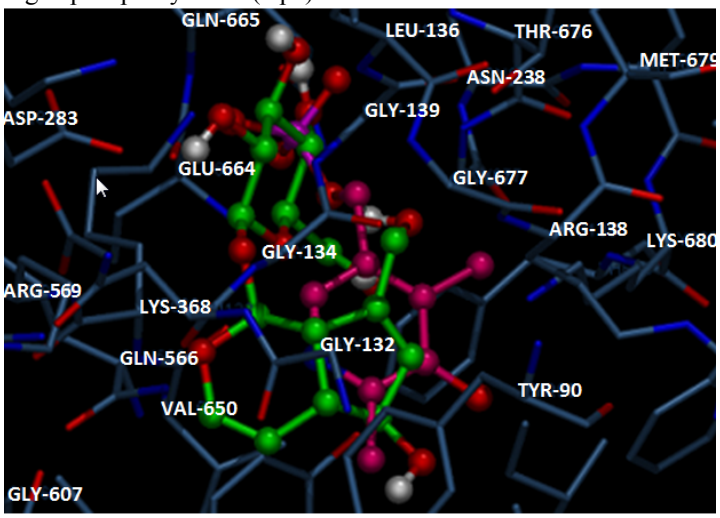

Figure 6. Docking poses of Aucubin (green) and the ligand PLP (pink) docked into the glycogen phosphorylase-a binding site (PDB code 1LWO).

Aucubin showed better docking energy (-7.38 Kcal/mol) than morroniside (-6.69 kcal/mol) in its binding affinity towards Gpa (Table 1). This is because glucose moiety in aucubin is taking part in binding as confirmed by two overlapping images of aucubin (green) and glucose (pink) at the PLP binding site of 1LWO (Figure 6). Moreover, morroniside and aucubin possess a different fused ring; a pyran ring for morroniside, and a cyclopentane ring for aucubin. The two fused rings exhibit different conformations, which in part is playing a role in the suggested activity.

A Novel Pharmacophore: Structure Activity Relationship for GPa

Based on the structural resemblance of iridoid and secoiridoid glycosides, we have designed a general pharmacophore structure (Figure 7) that may possess a better binding to GPa. A comparison of various iridoid and secoiridoid glycosides showed that the basic pyran ring was a must for binding to GPa. Based on docking experiments, we proposed structure activity relationship for inhibition of GPa enzyme as follows:

1. Substitution at position 4, with free carboxylic acid such as in loganic acid, showed a higher binding affinity and better docking energy (-7.78 kcal/mol) to GPa. Lower esters, such as in loganin, showed a lower binding affinity (-6.46 kcal/mol) toward Gpa. Furthermore, removal of the carboxylic acid group, such as in aucubin, also lowered the binding affinity towards GPa (-7.38 kcal/mol).
2. Unsaturation between carbon 3 and 4 is important for keeping the activity.

Saturation leads to loss of the desired pharmacological effects.

3. Presence or absence of unsaturation between carbons 6 and 7 in cyclopentanepyran ring e.g., Aucubin, Loganic acid, Loganin, does not drastically affect the binding activity (Table 1).
4. Substitution with hydroxy methyl at position 8 in cyclopentanepyran was found to be an effective substitution and increased binding affinity to GPa (Table 1). Moreover, such molecules have shown insulin releasing effect through regenerating β-cells of pancreas, e.g. Genipin and Aucubin (67). The absence of this group in loganin and loganic acid was compensated with the cumulative effect of the carboxyl and free hydroxyl groups.

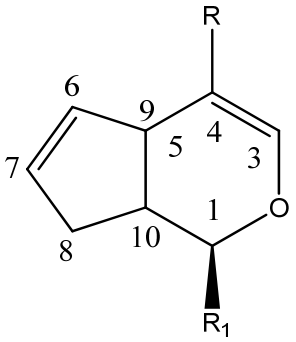

Figure 7. Proposed Pharmacophore Structure

CONCLUSIONS

Iridoid and secoiridoid glycosides exhibit a wide range of beneficial effects, e.g. cardiovascular benefits, hypoglycemic, hypolipidemic, and anti-inflammatory activities. Iridoid and secoiridoid glycosides were found to have increased affinity for GPa than α-D-glucose itself (k_i = 1.7 mM). The ligands were structurally similar in that they all possess the glucosyl moiety at position 1 but differ in the substituents attached to the pharmacophore moiety. To our knowledge, we are the first to report that GPa is a common molecular target, which is likely responsible for the anti-diabetic effects of these compounds. Our docking studies and structure activity relationship is a strong indication that GPa is a potential target for the treatment of diabetes.

ACKNOWLDGEMENT

Authors would like to thank the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC) for providing financial support. Hitesh Vaidya and Abeer Ahmed have an equal contribution towards this work

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract, 2010; 87:4-14.
2. DeFronzo RA, Bonadonna RC, Ferannini E. Pathogenesis of NIDDM: a balanced overview. Diabetes Care, 1992; 15:318–368.
3. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes, 1988; 37:1595-1607.
4. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes, 1992; 41:715-722.
5. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. Nature, 2001; 414:821-827.
6. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol, 2002; 81:81–100.
7. Bailey CJ, Day C. Metformin: Its botanical background. Pract Diab Inter, 2004; 21:115-117.
8. Dinda B, Debnath S, Y. H. Naturally Occurring Secoiridoids and Bioactivity of Naturally Occurring Iridoids and Secoiridoids. A Review, Part 2 Chem Pharm Bull, 2007; 55:689-728.
9. Wu SY, Wang GF, Liu ZQ, Rao JJ, Lu L, Xu W, et al. Effect of geniposide, a hypoglycemic glucoside, on hepatic regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. Acta Pharmacol Sin, 2009; 30:202-208.
10. Bianco A. The Chemistry of Iridoids" in "Studies in Natural Products Chemistry. Amsterdam: Elsevier Science Publishers;1990.
11. Bianco A. Recent developments in iridoids chemistry. Pure Appl Chem, 1994; 66:2335-2338.
12. Roth LM, Eisner T. Chemical Defenses of Arthropods. Ann Rev Entomo, 1962; 7:107.
13. Tundis R, Loizzo MR, Menichini F, Statti GA, Menichini F. Biological and pharmacological activities of iridoids: recent developments. Mini Rev Med Chem, 2008; 8:399-420.
14. Ghisalberti EL. Biological and pharmacological activity of naturally occurring iridoids and secoiridoids. Phytomedicine, 1998; 5:147-163.
15. Halpern O, Schmid H. Zur Kenntnis des Plumierids. 2. Mitteilung. Helv Chim Acta, 1958; 41:1109-1154.
16. El-Naggar LJ, Beal JL. Iridoids. A review. J Nat Prod, 1980; 43:649-707.
17. Junior P. Recent Developments in the Isolation and Structure Elucidation of Naturally Occurring Iridoid Compounds. Planta Med, 1990; 56:1-13.

18. Damtoft S, Franzyk H, Jensen SR. Biosynthesis of secoiridoids in Fontanesia. Phytochemistry, 1995; 38:615-621.
19. Foderaro TA, Stermitz FR, H. H. (5aH)-6-Epidihydrocornin, the First Known Iridoid Glycoside With a Trans-fused Ring System. Tetrahedron Lett, 1992; 33:2953-2954.
20. Boros CA, Stermitz FR. Iridoids, an up-dated review, Part II. J Nat Prod, 1991; 54:1173-1246.
21. Cornforth JW. The chiral methyl group--its biochemical significance. Chem Britain, 1970; 6:431-435.
22. Otsuka H. Two iridoid glycoside caffeoyl esters from Premna odorata. Phytochemistry, 1993; 33:617-622.
23. Inouye H, Uesato S. Biosynthesis of iridoids and secoiridoids. Prog Chem Org Nat Prod, 1986; 50:169-236.
24. Jensen J, Serup P, Karlsen C, Nielsen TF, Madsen OD. mRNA profiling of rat islet tumors reveals nkx 6.1 as a beta-cell-specific homeodomain transcription factor. J Biol Chem, 1996; 271:18749-18758.
25. Anwar M, Ahmad M, Aslam M, Aftab K. Enicostema littorale: a new source of swertiamarin. Pak J Pharm Sci, 1996; 9:29-35.
26. Vishwakarma SL, Rajani M, Goyal RK. Comparative antidiabetic activity of different fractions of Enicostemma littorale Blume. in STZ induced NIDDM rats. OPEM, 2003; 3:196-204.
27. Vishwakarma SL, Rajani M, Bagul MS, Goyal RK. A rapid method for the isolation of swertiamarin from Enicostemma littorale. Pharm Biol, 2004; 42:400-403.
28. Vaidya H, Mandapati R, Vasudevan S, Padh H, Goyal RK. Antihyperlipidaemic activity of swertiamarin, a secoiridoid glycoside in poloxamer-407-induced hyperlipidaemic rats. J Nat Med, 2009; 63:437-442.
29. Vaidya H, Mandapati R, Vasudevan S, Padh H, Goyal RK. Swertiamarin: A lead from Enicostemma littorale Blume. for anti-hyperlipidaemic effect. Eur J Pharmacol, 2009; 617:108–112.
30. Vaidya H, Prajapati A, Rajani M, Sudarsanam V, Padh H, Goyal RK. Beneficial Effects of Swertiamarin on Dyslipidaemia in Streptozotocin-induced Type 2 Diabetic Rats. Phytother Res, 2012; 26:1259-1261.
31. Vaijanathappa J, Badami S. Antiedematogenic and free radical scavenging activity of swertiamarin isolated form Enicostemma axillare. Planta Med, 2009; 75:12-17.
32. Danbuo, Y. editor. Zabing Guagyao. 2 ed. China: People's Health Publishers, PR China; 1984.
33. Wang, X. editor. Zhongyi Neike Bianzheng Xue. 1 ed. China: P. R. China: People's Health Publishers; 1991.
34. Krauss S, Zhang CY, Lowell BB. A significant portion of mitochondrial proton leak in intact thymocytes depends on expression of UCP2. . Proc Natl Acad Sci USA, 2002; 99:118-122.
35. Chen-Yu Z, Laura EP, Chian Ping Ye, Krauss S, Ruichao Shen, Cheng-Ting Lin, et al. Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced β cell dysfunction in isolated pancreatic islets. Cell Metab, 2006; 3:417-427.
36. Stupans I, Kirlich A, Tuck KL, Hayball PJ. Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants. J Agric Food Chem, 2002; 50:2464-2469.
37. Visioli F, Bellomo G, Galli C. Free radical scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun, 1998; 247:60–64.
38. Visioli F, Bellomo G, Montedoro G, Galli C. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis, 1995; 117:25–32.
39. De lCJ, Quintero L, Villalobos MA, Sanchez dl, Cuseta F. Lipid peroxidation and glutathione system in hyperlipemic rabbits: influence of olive oil administration. Biochim Biophys Acta, 2000; 1485:36-44.
40. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, et al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol, 2003; 23:622-629.
41. Kris-Etherton PM, Hecke KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med, 2002; 113:71S–88.
42. Speroni E, Guerra MC, Minghetti A, Crespi-Perellino N, Pasini P, Piazza F. Oleuropein evaluated in vitro and in vivo as an antioxidant. Phytother Res, 1998; 12:S98-100.
43. Chimi H, Cillard J, Cillard P, Rahmani M. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. J Am Oil Chem Soc, 1991; 68:307–312.
44. Hedya Jemai, Abdelfattah El Feki, Sayadi S. Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats. J Agric Food Chem, 2009; 57:8798–8804.
45. Gonzalez M, Zarzuelo A, Gamez MJ, Utrilla MP, Jimenez J, Osuna I. Hypoglycemic activity of olive leaf. Planta Med, 1992; 58:513–515.
46. Calis I, Zor M, Basaran AA, Karsoside. Scropolioside D, Two New iridoid Glycosides from

Scrophularia ilwensis. J Nat Prod, 1993; 56:606-609.
47. Bhandari SP, Mishra A, Roy R, Garg HS. Koelzioside, an iridoid diglycoside from Scrophularia koelzii. Phytochemistry, 1992; 31:689-691.
48. Perry, LM; Metzger, J, editors. Medicinal Plants of Southeast Asia: MIT Press, Cambridge, MA and London, UK 1980.
49. Shabana MM, Mirhom YW, Genenah AA, Aboutabl EA, Amer HA. Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. Arch Exp Veterinarymed, 1990; 44:389-394.
50. Fan C, Xiang QY. Phylogenetic relationships within cornus (cornaceae) based on 26s rdna sequences-1. Am J Bot, 2001; 88:1131-1138.
51. Yamabe N, Kang KS, Goto E, Tanaka T, Yokozawa T. Identification of Antidiabetic Effect of Iridoid Glycosides and Low Molecular Weight Polyphenol Fractions of Corni Fructus, a Constituent of Hachimi-jio-gan, in Streptozotocin-Induced Diabetic Rats. Biol Pharm Bull, 2007; 30:520-526.
52. Cooper ME, Bonnet F, Oldfield M, Jandeleit-Dahm K. Mechanisms of diabetic vasculopathy: an overview. Am J Hypertens, 2001; 14:475-486.
53. Xu HQ, Hao HP. Biological Effects of Iridoid Total Glycoside from Cornus officinalis on Prevention of Glomerular Overexpression of Transforming Growth Factor Beta 1 and Matrixes in an Experimental Diabetes Model. Biol Pharm Bull, 2004; 27:1014-1018.
54. Pailer M, Haschke Hofmeister E. Inhaltsttoffe aus Plantago major. Planta Med, 1969; 17:139-145.
55. Bianco A, Iavarone C, Trogolo C. Structure of eucommiol, A new cyclopentenoid-tetraol from Eucommia ulmoides. Tetrahedron, 1974; 30:4117–4121.
56. Bernini R, Iavarone C, Trogolo C. 1-O-β- -Glucopyranosyleucommiol, an iridoid glucoside from Aucuba japonica. Phytochemistry, 1984; 23:1431–1433.
57. Suomi J, Sire´n H, Wiedmer SK, Riekkola ML. Isolation of aucubin and catalpol from Melitaea cinxia larvae and quantification by micellar elec trokinetic capillary chromatography. Anal Chim Acta, 2001; 429:91-99.
58. Sampio-Santos MK, M. . Biosynthesis Significance of iridoids in chemosystematics. J Braz Chem Soc, 2001; 12:144-153.
59. Lei JH-Y, Xue.; Li-Ji, Jin.; Shu-Ying, Li.; Yong-Ping, Xu. . Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. Eur J Pharmacol, 2007; 583:162-167.
60. Hong YX, LiJi J, Lei J, Z. P, DanQing L, YanQiu X, et al. Neuroprotection of aucubin in primary diabetic encephalopathy. Science in China Series C: Life Sci, 2008; 51:496-502.
61. Newgard CB, Hwang PK, Fletterick RJ. The family of glycogen phosphorylases: structure and function. Crit Rev Biochem Mol Biol, 1989; 24:69-99.
62. Rath VL, Ammirati M, Danley DE, Ekstrom JL, Gibbs EM, Hynes TR, et al. Human liver glycogen phosphorylase inhibitors bind at a new allosteric site. Chem Biol, 2000; 7:677-682.
63. Hazai E, Kovacs S, Demko L, Bikadi Z. DockingServer (www.dockingserver.com) Virtua Drug Ltd., Budapest, Hungary [cited 2012 Jan 2012].
64. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comp Chem, 1996; 17:490-519.
65. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comp Chem, 1998; 19:1639-1662.
66. Francisco J. Solis, Wets RJ-B. Minimization by Random Search Techniques. Math oper res, 1981; 6:19-30.
67. Zhang CY, Parton LE, Ye CP, Krauss S, Shen R, Lin CT, et al. Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced beta cell dysfunction in isolated pancreatic islets. Cell Metab, 2006; 3:417-427.