

**AN OVERVIEW OF EMERGING PHARMACOLOGICAL TARGETS IN TREATMENT OF TYPE 2 DIABETES MELLITUS**

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Received 28 June 2013; Revised 07 July 2013; Accepted 10 July 2013**ABSTRACT**

Diabetes is a chronic disorder which has serious complications if not treated appropriately. A number of therapeutic agents exist for the treatment of type 2 diabetes including metformin, sulfonylureas, thiazolidinediones, α -glucosidase inhibitors, insulin, GLP-1 analogs and DPP-4 inhibitors. Despite a laborious list, they still suffer from few of the adverse effects like hypoglycemia, weight gain, edema, fractures, lactic acidosis etc. The objective of this article is to review the important drug classes currently in the development for the treatment of diabetes. Many new classes of drugs are in different stages of development which have novel mechanism of action and have shown promising results. These drugs mainly target the kidney, liver and pancreas as their prime target in bringing about glucose control. These new classes of drugs include Sodium-Glucose Cotransporter-2 inhibitors, Peroxisome proliferator activated receptor- α agonists, Fructose-1,6-bisphosphatase Glycogen phosphorylase inhibitors, Glucokinase activators, G-Protein coupled receptor 119 agonists, Glycogen synthase kinase-3 inhibitors, Protein tyrosine phosphatase-1b inhibitors, AMP activated protein kinase activators, Liver selective glucocorticoid receptor antagonist (LSGRA), 11β -hydroxysteroid dehydrogenase Type-1 inhibitors, JNK activators, Free fatty acid receptor-1 (FFAR-1) agonists. Further exploration of these new targets for glycemic control will hopefully lead to development of safe and efficacious drugs that will become the mainstay of next generation therapeutics for diabetes and will reduce the clinical and cost burden of this disease.

KEY WORDS: Diabetes mellitus, emerging targets, current medications, gluconeogenesis, glycogenolysis.

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbance in the carbohydrate, fat and protein metabolism resulting from altered insulin secretion and/or action (insulin resistance).¹ There may be associated glycosuria, negative nitrogen balance or ketonaemia. It is a progressive disease resulting in complications like nephropathy, retinopathy, neuropathy, and vascular complications. Diabetes is one of the major causes of heart disease and stroke and is the seventh leading cause of death among the US adults.²

It is rapidly increasing in prevalence and is a major public health problem with estimated prevalence of 6% in 2007 and is expected to rise to 7.3% in 2025. It incurs a significant burden to the individual and also to the country's economy.³ The prevalence of diabetes is expected to grow from 171 million patients worldwide in the year 2000 to 366 million patients (30 million in the United States alone) by 2030.⁴

India contains the largest number of diabetic population across the world and WHO estimates it at 32 million people in 2000 and is expected to rise to 80 million people by 2030. The prevalence is increasing in urban areas from 2% in 1970s to 12% in 2000 and rural areas are also now showing an increase.⁵

Mechanism of action of available drugs includes increasing insulin secretion, increasing insulin sensitivity, controlling hepatic glucose release or inhibiting intestinal glucose absorption. Current national and international guidelines advocate use of metformin, alongside diet and lifestyle measures as initial pharmacotherapy followed by additional oral therapy and finally insulin. However, with this strategy there is no effect on the declining beta cell function. Because of the limitations with the currently available anti-diabetes agents, there is a need for newer therapies with the qualities of low risk of hypoglycemia, and lack of weight gain and ideally which also will improve beta cell function.

DATA SEARCH METHODOLOGY

This included manual search as well as electronic search of the databases of publications and cross references. Electronic search included Pubmed, Google, etc. Electronic search of cross references yielded other relevant materials. Terminologies used for search in electronic databases included diabetes mellitus, emerging targets, pipeline drugs, novel molecules, preclinical development, clinical trials, recent advances, and management.

CRITICAL APPRAISAL OF CURRENT ANTI-DIABETIC MEDICATIONS AND NEED FOR NEW DRUGS:

Tight glycemic control has been the need of the hour in the treatment of diabetic patients and presently more emphasis is laid on comprehensive diabetes care which includes management of diabetes related complications also. The number of therapeutic agents available for the treatment of type 2 diabetes mellitus (T2DM) includes metformin, sulfonylureas, biguanides, thiazolidinediones, DPP-4 inhibitors, α -glucosidase inhibitors, insulin and GLP-1 analogs. For many years, the only therapeutic option for type 2 diabetes was insulin and sulfonylureas however, over the past 10-12 years there has been new and exciting agents approved for type 2 DM. Some of them treat insulin deficiency or insulin resistance and some retard intestinal carbohydrate absorption. With insulin therapy, there is incidence of hypoglycemia, weight gain, edema (high doses) and increased risk of atherogenesis.³

Sulfonylureas (SU) are effective in target reduction of HbA_{1c} but they carry the major limitation of hypoglycemia and weight gain which is seen more with gliburide whereas glipizide and glimepiride are more weight neutral. More recent trials have refuted the claims of cardiovascular risk associated with use of SU. Widely used biguanides namely metformin reduce fasting plasma glucose, improve lipid profile and have modest weight loss, but cause gastrointestinal (GI) disturbances and lactic acidosis in some patients. Thiazolidinediones cause hepatic injury, increased risk of fracture and heart failure. Meglitinide analogues may cause hypoglycemia if meal is skipped and α -glucosidase inhibitors cause GI intolerance like flatulence and diarrhea.³ The newer drugs namely GLP-1 analogs and DPP-4 inhibitors show reduced incidence of hypoglycemia due to their glucose dependent insulin secretion.⁶ Exenatide has shown post-marketing reports of pancreatitis⁷ and incidence of angioedema, anaphylaxis and rashes like Steven Johnson syndrome has been reported with Sitagliptin.

Common to most of available drugs is that they neither alter nor retard the progression of diabetes or

related complications. Hence the requirement is focused on improving glucose control and proven benefit in improving the macro and/or microvascular complications like nephropathy, neuropathy or retinopathy. Since presently, >30 drugs from 9 different classes are available and no urgency exists, the regulatory aspects should be made more stringent in the development of novel molecules with more emphasis on demonstration of safety of these molecules on CVS outcomes.

EMERGING TARGETS:

SODIUM-GLUCOSE COTRANSPORTER-2 (SGLT2) INHIBITORS:

Kidneys play an important role in filtration and reabsorption of glucose. They contribute to glucose balance by producing glucose through gluconeogenesis,⁸ utilizing glucose in renal medulla⁸ and nearly 100% reabsorption of filtered glucose.⁹ Glucose is a polar molecule and its transport across the cell membrane is accomplished by membrane associated carrier proteins, facilitative glucose transporters (GLUTs) and Na⁺-coupled glucose co-transporters (SGLTs).² Two SGLT isoforms have been identified: SGLT1 is expressed in heart, gastrointestinal tract, liver, skeletal muscle, lung and S3 segment of proximal tubule, where it accounts for <10% of glucose reabsorption.¹⁰ SGLT2 is expressed almost exclusively in the S1 segment of the proximal tubule and accounts for >90% of glucose reabsorption. Studies have shown that there is increased expression of SGLT2 in diabetic patients.¹¹ Hence, selective inhibition of this transporter will decrease the renal reabsorption of the filtered glucose, thus lowering the plasma glucose concentration and improving glycemic control. Several SGLT2 inhibitors have been developed which are in various phases of clinical trial.

Dapagliflozin

The most advanced agent in this class. It has been tested in phase 3 trials in patients with Type 2 diabetes as monotherapy,¹² or in combination with other agents i.e metformin,¹³ glimepiride,¹⁴ insulin or insulin plus other anti-diabetic agents.¹⁵ The drug has been well tolerated with most common adverse reactions being urinary tract infection, dizziness, headache, fatigue, back pain and nasopharyngitis. In a 12 week, randomized, double blind, placebo controlled study enrolling 389 treatment naïve patients with Type2 diabetes, Dapagliflozin doses from 2.5 to 50mg once daily were associated with greater reduction in HbA1C than placebo (-0.55% to -0.9% Vs -0.18%; P<0.01). At doses from 5-50mg once daily, fasting plasma glucose reduced more with Dapagliflozin group as compared to placebo (-19.3 to -30.5 Vs -5.8 mg/dl; P <

0.01). Urinary glucose excretion ranged from 51.8 to 85g/day at week12 in Dapagliflozin arm compared to 5.8 to 10.9g/day at baseline and 5.7g/day at week 12 in placebo group. The mean weight loss was more in Dapagliflozin group compared to placebo group.¹⁶ Currently, it is already approved in Europe in November 2012 but, the US-FDA has issued a complete response letter for better assesment of risk benefit profile since an increased risk of breast and bladder cancer was observed in patients taking this drug. But, still this group of drugs holds promise to be added to the list of medications for management of patients with type 2 DM.¹⁷

Canagliflozin First drug in this class to be approved as oral inhibitor of SGLT2 in March, 2013. In a phase III study, canagliflozin was compared with placebo as add on therapy to metformin and pioglitazone in adult patients with type 2 DM. The results of the study (known as DIA 3012) showed that Canagliflozin, dosed once daily at 100mg or 300mg in addition to metformin and pioglitazone, had statistically greater HBA_{1c} reduction at 26weeks relative to placebo (change from the baseline , -0.89% and -1.03%, Vs -0.26%, respectively, p<0.001). The overall incidence of adverse events was generally similar across all treatment arms.

In secondary efficacy endpoint measures of the study, both the canagliflozin 100 mg and 300 mg dose groups provided reductions in body weight compared to placebo (-2.8% and -3.8% vs. -0.1%, respectively, p<0.001) and reductions in systolic blood pressure (-5.3 mm Hg, p<0.01and -4.7 mm Hg, p<0.05, vs. -1.2 mm Hg, respectively). Reductions in fasting plasma glucose were consistent with the primary endpoint for canagliflozin 100 mg and 300 mg, compared to placebo (-1.49 and -1.84mmol /L Vs 0.14 mmol /L, respectively, p<0.001). Increases in high-density lipoprotein cholesterol were observed with canagliflozin 100 mg and 300 mg, compared to placebo [7.2% (0.08 mmol /L), p<0.05, and 8.9% (0.10 mmol /L), p<0.001, vs. 2.4% (0.02 mmol /L), respectively], and increases in low-density lipoprotein cholesterol were also seen [7.1% (0.08 m mol /L) and 11.3% (0.19 mmol /L) vs. -0.4% (-0.10 mmol /L), respectively]. Canagliflozin decreased triglyceride levels at the 300 mg dose [-1.7% (-0.16 mmol /L), p<0.01]. Triglyceride levels increased at the 100 mg dose [3.1% (-0.06 mmol/L), p=ns], though placebo was associated with a higher increase [15.3% (0.10 mmol/L)].¹⁸ Others in this class include sergliflozin (phase2), empagliflozin, ipragliflozin and YM-543(phase2).

PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS:

These receptors belong to nuclear receptor superfamily that includes retinoic acid receptors (RARs), thyroid hormone receptors and steroid receptors and is ubiquitously expressed throughout in the body. Three major types of PPARs have been identified till date namely, PPAR α , PPAR β/δ and PPAR γ .

Molecular mechanism

Following the binding of ligand, PPARs undergo conformational change and heterodimerize with retinoid X receptor (RXR). This complex binds to specific regions on the DNA of target gene called the PPAR response elements (PPRE) in the promoter region and transcription of the genes are increased or decreased depending on the gene. The function of the PPAR is modified by the precise shape of the ligand binding domain and a number of coactivator and corepressor proteins, the presence of which can stimulate or inhibit the receptor function respectively.¹⁹

Physiological role of PPAR receptors

PPAR α

They are mainly related to lipid metabolism. They are expressed in both rodents and humans and are found in various tissues like liver, kidney, heart, skeletal muscle and brown fat and also in vascular endothelial, smooth muscle cells and in monocytes/macrophages. Palmitic acid, stearic acid, arachidonic acid etc are endogenous ligands whereas fibrates like gemfibrozil and nafenopin are exogenous ligands. Activation of these receptors causes increased lipoprotein lipase activity, decreased triglyceride secretion from liver and increased β -oxidation in liver leading to increased HDL and reduced TGs and FFAs, enhancement of reverse cholesterol transport, reduction in vessel wall inflammation.²⁰

PPAR β

Their functional identity is not known. However, they are found to be implicated in dyslipidemia, insulin resistance, hyperlipidemia, inflammation, atherosclerosis, obesity, infertility, cancer, and nervous system.²¹

PPAR γ

They are of three subclasses namely PPAR γ 1, PPAR γ 2 and PPAR γ 3. PPAR γ 1 is found in wide range of tissue, PPAR γ 2 is found in adipose tissue and PPAR γ 3 is found in macrophages, intestine, adipose tissue and heart. Endogenous ligands are arachidonic acid and linolenic acid and exogenous ligands are thiazolidindiones. Agonists improve insulin sensitivity, reduce the plasma glucose and A1C, raise the HDL and reduce the LDL levels. PPAR γ agonists oppose the action of TNF- α , a proinflammatory cytokines associated with insulin resistance.²²

PPAR α/γ dual agonists

PPAR γ activation causes insulin sensitization and enhances glucose metabolism and PPAR α activation is associated with reduction of triglycerides and energy

homeostasis. PPAR α mitigates the weight gain mediated through PPAR γ activation. Idea with this dual agonistic activity came into forefront, as large percentage of population has both the elements namely, hyperglycemia and hyperlipidemia.

Muraglitazar is a dual agonist and in 2006 the clinical trial was discontinued after it was found to have increased incidence of cardiovascular events (MI, congestive heart failure, cerebral vascular events and transient ischemic attacks) when compared to placebo or pioglitazone. Clinical development of tesaglitazar was discontinued due to renal toxicity. Another PPAR α / γ dual agonist aleglitazar which is in phase II randomized, double blind, multicentric study involving type 2 diabetic patients (either treatment naïve or pretreated with \leq two oral agents), after a 4-5 week placebo run-in period, 332 patients were randomized to receive 16 weeks treatment with aleglitazar once daily doses of 50, 150, 300 and 600 or placebo (n=55 in each group) or to open label pioglitazone 45mg once daily (n=57) as reference.

Aleglitazar significantly reduced baseline HbA1c versus placebo in a dose-dependent manner, from -0.36% (95% CI 0.00 to -0.70, p=0.048) with 50 μ g to -1.35% (-0.99 to -1.70, p<0.0001) with 600 μ g. Oedema, haemodilution and weight gain occurred in dose dependent manner. At doses less than 300 mg, none of the patients had congestive heart failure and frequency of oedema was similar to placebo but less when compared to Pioglitazone group and bodyweight gain was also less than with pioglitazone (0.52 kg at 150 μ g vs 1.06 kg). Aleglitazar significantly reduced the TG/HDL ratio vs placebo. Aleglitazar is now being studied in a large scale to assess the cardiovascular endpoints (death, myocardial infarction and stroke) among patients with diabetes and coronary artery disease (ALECARDIA trial). If the studies find this drug to be safe, then aleglitazar may become the first therapy to reduce macrovascular complications in patients with diabetes.²³

FRUCTOSE-1,6-BIPHOSPHATASE INHIBITORS:

Uncontrolled blood glucose levels or hyperglycemia over long term leads to micro- and/or macro vascular complications. In type 2 diabetic patients there is increased endogenous glucose production (EGP) and liver is the main organ responsible for the same. Glucose is produced by liver by two pathways namely, gluconeogenesis (synthesis of glucose from alanine, glycerol and lactate) or glycogenolysis (breakdown of liver glycogen). In healthy individuals gluconeogenesis accounts for 50% EGP after overnight fast and 90% of EGP after 96 hrs of fasting. Fructose 1,6 biphosphatase is a key enzyme in gluconeogenesis and mediates the conversion of fru-1,6

biphosphate to fru-6-phosphate. In patients with type 2 diabetes, the rate of gluconeogenesis is increased during fasting, whereas the rate of glycogenolysis is either unchanged or reduced. Thus in type 2 diabetic patients increased gluconeogenesis is primarily responsible for increased EGP during fasting, whereas inappropriate rates of both glycogenolysis and gluconeogenesis accounts for increased post prandial EGP. FBP inhibitors are an emerging class of antidiabetic drugs which interfere with endogenous glucose production.¹⁹

CS-917

CS-917, a Fructose-1,6-biphosphatase inhibitor, in Phase IIb trials has showed negative results for the treatment of type II diabetes. This multi-center, double-blind, placebo-controlled trial enrolled 392 subjects who were divided into four treatment arms to receive CS-917 at 50 mg BID or 100 mg BID, metformin at 850 mg BID or placebo, for three months. Both doses of CS-917 failed to achieve statistical significance in lowering the placebo-adjusted level of glycosylated hemoglobin (HbA1c), which is a measure of glucose load. The mean HbA1c level at the start of the study was approximately 7.6-7.7%. The placebo adjusted HbA1c level at the end of treatment was unchanged at the CS-917 low dose and there was a 0.17% decrease at the high dose (p=0.1256). Metformin treatment resulted in a placebo-adjusted decrease of HbA1c by 0.50% (p < 0.0001).²⁴

GLYCOGEN PHOSPHORYLASE INHIBITORS:

Liver plays a central role in glucose handling, homeostasis and accounts for nearly 90% of endogenous glucose production. In diabetic patients there is excessive glucose production coupled with insulin resistance, responsible for hyperglycemia. As mentioned above, liver produces glucose either by gluconeogenesis or glycogenolysis. Glycogen phosphorylase is a key enzyme in glycogenolysis which catalyses the phosphorolytic cleavage of glycogen to produce glucose-1-phosphate, which then enters glycolytic pathway to produce glucose.²⁵ In animal models of diabetes or insulin resistance, hepatic activity of glycogen phosphorylase is increased; inhibitors that bind and inactivate this enzyme reduce glycaemia in animal models of diabetes. Creating an inhibitor that specifically targets glycogen phosphorylase would then essentially decrease the amount of glucose produced by the liver. There are 5 binding sites on the glycogen phosphorylase enzyme that have been found to be potential targets—the catalytic site, inhibitor site, adenosine monophosphate (AMP) allosteric site, glycogen storage site, and a new allosteric binding site.²⁶

Glycogen phosphorylase exists in two interconvertible forms namely glycogen phosphorylase 'a',

the more active form and glycogen phosphorylase 'b', the less active form. The proportion that exists in each form is regulated by phosphorylation reaction mediated by glycogen phosphorylase kinases.

Martin and colleagues found that CP-91149, which has been characterized in vitro and in vivo, suppressed glycogenolysis in both rat and human liver cells. When studying obese mice, the investigators found that a single 50mg/kg oral dose of CP-91149 reduced plasma glucose concentrations to near-normal levels 3 hours after administration (plasma glucose 235 ± 21 mg/dL with vehicle vs 134 ± 7 mg/dL with CP-91149).²⁷ Oikonomakos and colleagues found that CP- 320626 to be an inhibitor of the human liver glycogen phosphorylase by binding at the allosteric site compared with the inhibitor site that CP-91149 binds to.²⁸

GLUCOKINASE ACTIVATORS:

Glucokinase or hexokinase IV is a predominant hexokinase expressed in the liver and pancreas, and plays an important role in glucose homeostasis. It acts as a glucose sensor in the pancreatic beta cells and serves as a rate-controlling enzyme for hepatic glucose clearance and glycogen synthesis. Both of these processes are impaired in T2DM.²⁹ The glucose-phosphorylation enzyme glucokinase, is a promising target for developing new anti-diabetic agents. This is supported by the fact that inactivating mutations of GK gene, lower the enzyme's affinity for glucose or compromise glucokinase expression thereby causing diabetes (maturity onset diabetes of the young type), whereas activating mutations lower blood glucose in humans.³⁰ Hence, a pharmacological activator may be a novel and a potential agent for the treatment as an anti-hyperglycemic.

In the pancreas, glucokinase governs the beta-cell threshold for glucose stimulated insulin release. High glucose increases GK expression in beta-cells as much as 5- to 10-fold, in a concentration-dependent manner, and thus sensitizes them to glucose stimulated insulin biosynthesis and release. 99% of the body's GK protein resides in the liver regulated by glucokinase regulatory protein (GKRP), plays critical role in post-prandial clearance of glucose from blood stream coupled with enhanced glycogen synthesis and glucose catabolism.³¹

GK possesses an allosteric to which bind the glucokinase activators (GKAs), including GKAs 1 through 14 thereby, leading to increase in the enzymatic activity of glucokinase. One possible side effect of GKAs is that they can induce moderate hypoglycemia because they increase the amount of insulin release.³² Thus glucokinase looks a promising target for development of new molecule for treatment of type2 diabetes due to the predominant role it

plays in glucose homeostasis in liver and as a glucose sensor, its role in insulin release from pancreas.

G PROTEIN-COUPLED RECEPTOR 119 AGONIST:

G protein-coupled receptor 119 has attracted considerable interest as a T2DM drug target in recent years. The activation of GPR119 increases the intracellular accumulation of cAMP, leading to enhanced glucose-dependent insulin secretion from pancreatic β -cells and increased release of the gut peptides GLP-1 (glucagon-like peptide 1), GIP (glucose-dependent insulinotropic peptide) and PYY (polypeptide YY). In humans, it is encoded by GPR119 gene. GPR 119 consists of two endogenous ligands: phospholipids and fatty-acid amides (Overton et al.,2006; Soga et al., 2005). GPR119 acts by coupling to G protein α -subunit $G\alpha/s$ and increasing intracellular cAMP levels.³³ Chu et al. described a new agonist for GPR119, AR231453, which was found to enhance glucose stimulated insulin release (GSIS) and improve glucose tolerance in both normal and diabetic mice. Interestingly, the drug did not affect feeding behavior at doses that are effective in normalizing glucose tolerance.³⁴

Surprisingly, Chu and colleagues showed that oral treatment with a GPR119 agonist AR231453 provided better glycemic control than intravenous treatment, which suggests possible incretin involvement.³⁵ Further more, Flock and colleagues demonstrated that AR231453 not only directly increases insulin, incretin, and GLP-1 levels but also, independently of incretins, slows gastric emptying. Adding to these results, Yoshida and colleagues confirmed that the GPR119 agonist AS1907417 is effective in preserving beta-cells and controlling glucose levels in HEK 293 cells expressing human GPR119.³⁶ Overall, GPR119 agonists, by activating several complementary pathways, may render effective glucose control in patients with type 2 diabetes. Thus, the development of a compound that can be administered orally to improve glucose tolerance and reduce the body weight by controlling the food intake may represent a major improvement over currently available therapies but, further investigations are required to tell if GPR 119 agonists would meet this goal.

GLYCOGEN SYNTHASE KINASE-3 INHIBITORS:

Glycogen synthase kinase-3 (GSK3), is a constitutively acting multi-functional serine threonine kinase involved in diverse physiological pathways ranging from glycogen metabolism, insulin signalling, cell cycle, gene expression, development and oncogenesis to neuroprotection. GSK3 has been implicated in various diseases such as diabetes, inflammation, cancer, alzheimer's and bipolar disorder. GSK3 negatively regulates insulin-mediated glycogen synthesis and glucose homeostasis by phosphorylation and inhibition of glycogen

synthase enzyme, and increased expression and activity of GSK3 has been reported in type II diabetics and obese animal models. Consequently, inhibitors of GSK3 have been demonstrated to have anti-diabetic effects *in vitro* and in animal models. However, inhibition of GSK3 poses a great challenge, because of its involvement in various pathways where its inhibition may lead to side effects and toxicity.³⁷

The key enzyme for glycogen synthesis is glycogen synthase which mediates the production of glycogen from glucose. Those drugs which inhibit the GSK3 enzyme would promote the glycogen synthesis and reduce the plasma glucose levels.

GSK3 inhibitors for type2 DM: Lithium is an ATP-noncompetitive GSK3 inhibitor. Inhibition of GSK3 by lithium stimulates glucose uptake, glycogen synthesis and normalizes insulin sensitivity in diabetic rats (Rossetti, 1989). As it is a non-selective inhibitor, it also inhibits other enzymes like CK2, p38-regulated/activated kinase and MAPK-activated protein kinase-2 (Phiel and Klein, 2001). Lithium also inhibits polyphosphate 1-phosphatase and inositol monophosphate (Phiel and Klein, 2001). The anti-diabetic effect of GSK3 is exerted by enhancing glucose disposal and improving insulin resistance. Apart from this, the recently demonstrated beneficial effects on β -cells provide a distinct advantage over other anti-diabetic therapies. GSK3 inhibitors could also have a beneficial effect for type I diabetes and also islet transplantation patients. Several potent GSK3 inhibitors have been identified and characterized in preclinical models for treatment against T2DM.³⁷

PROTEIN TYROSINE PHOSPHATASE-1B INHIBITORS:

Protein tyrosine phosphatase-1b is an enzyme which is expressed in nearly all the tissues of the body and is localized primarily on intracellular membrane. It negatively regulates the intracellular signalling of insulin by causing dephosphorylation of insulin receptors. It is shown that PTP-1b gene knockout in mice showed enhanced sensitivity to insulin and also opposed development of obesity.

Since PTP-1b shares 50-80% homology with other phosphatases like CD45, SHP-2, cdc25c and T-cell PTP, it was required to target a site outside the catalytic site in order to increase the selectivity and reduce the toxicity of the drug. A non-catalytic phosphotyrosine binding site was identified lying close to the catalytic site but less homologous in the amino acid sequences when PTP of different cells were compared. Hence, drug molecules showing their activity against this non-catalytic site may enhance their selectivity and activity.

Various PTP-1b inhibitors have been identified through high-throughput screening. Pyridazine derivatives,

hydroxyphenylazole derivatives, azolidinediones and phenoxyacetic acid based inhibitors and more recently pyrimidotriazinepiperidine analogues have been identified.³⁸

Role of PTP-1b inhibitors in diabetes associated with obesity

Leptin promotes weight loss, regulation of appetite and can reverse diabetes by improving glucose tolerance. PTP-1b negatively regulates insulin receptor signalling by dephosphorylating leptin Janus kinase (jak)-2. It was observed that leptin gene knockout (KO) mice and a control group of mice with intact leptin receptors were placed on a high-fat diet. Although both the control and KO mice became obese, only the KO mice developed severe glucose intolerance and insulin resistance, a precursor to the development of diabetes. These novel results indicate that in the presence of obesity, the combination of insulin resistance in the beta cell and the lack of leptin signaling leads to poor beta cell growth and function leading to glucose intolerance. This finding opens up new avenues for studying leptin and its role in islet cell biology, which may lead to new treatment for diabetes.³⁹

AMP ACTIVATED PROTEIN KINASE (AMPK) ACTIVATORS:

It is a heterotrimeric enzyme composed of catalytic subunit α and two regulatory subunits β and γ . Catalytic subunit α is present in two isoforms namely $\alpha 1$ and $\alpha 2$ of which AMPK $\alpha 1$ is widely distributed and AMPK $\alpha 2$, which is expressed in heart, liver and skeletal muscle. AMPK regulates many of the intracellular functions like cellular uptake of glucose, β oxidation of fatty acids, biogenesis of GLUT4. It is activated by decreases in ATP:ADP ratio, phosphocreatine (PCr)/creatin ratios through mechanisms involving phosphorylation by one or more upstream AMP kinases. The increase in AMPK activity results in the stimulation of glucose uptake in muscle, fatty acid oxidation in muscle and liver, and the inhibition of hepatic glucose production, cholesterol and triglyceride synthesis, and lipogenesis. Chemical activation of AMPK with the compound 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) leads to increased glucose uptake concomitantly with GLUT4 (15–19) and enhanced insulin sensitivity in muscle.^{41,42} In liver cells, activation of AMPK with AICAR causes inhibition of glucose production. AMPK induced glucose transport occurs through mechanism distinct to that used by classical insulin signalling pathway. Finally, AMPK enhanced glucose transport in skeletal muscle is observed both in rodents and in humans even if insulin resistance is present suggesting that muscular AMPK could be a therapeutic target for management of insulin resistance.

Metformin: Association with AMPK

Hypoglycemic action of metformin is through decreased hepatic glucose production and increased glucose disposal through skeletal muscle. AMPK activity increases in response to depletion in the cellular energy stores and this enzyme is implicated in stimulation of glucose uptake by skeletal muscle and inhibition of hepatic gluconeogenesis. Recent study suggests that AMPK is activated by metformin in cultured rat hepatocytes mediating its inhibitory effect on hepatic gluconeogenesis.⁴²

LIVER SELECTIVE GLUCOCORTICOID RECEPTOR ANTAGONISTS (LSGRA):

Glucocorticoids are steroidal hormones which play an essential role in glucose homeostasis. The primary metabolic target organ for glucocorticoid action is liver where glucocorticoids activate the transcription of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Barthel and Schmolli, 2003). Evidence showed that treatment of obese mice with RU-486 (Mifepristone), a systemic glucocorticoid receptor (GR) antagonist for 21 days normalized post prandial glucose values and reduced hyperglycemia. This approach of systemic glucocorticoid receptor antagonism can result in symptomatic adrenal insufficiency, as well as increased levels of circulating cortisol due to counter regulatory activation of hypothalamic pituitary adrenal axis. Hence, liver selective glucocorticoid receptor antagonist (LSGRA) may be therapeutic value.⁴³

A-348441 is a novel liver targeted GR antagonist with significant anti-diabetic activity. Antagonizes glucocorticoid-up-regulated hepatic genes, normalizes postprandial glucose in diabetic mice, and demonstrates synergistic effects on blood glucose in these animals when co-administered with an insulin sensitizer. In insulin-resistant Zucker *fa/fa* rats and fasted conscious normal dogs, A-348441 reduces hepatic glucose output (HGO) with no acute effect on peripheral glucose uptake. A-348441 has no effect on the hypothalamic pituitary adrenal axis or on other measured glucocorticoid-induced extra hepatic responses. Overall, A-348441 demonstrates that an LSGRA is sufficient to reduce elevated HGO and normalize blood glucose and may provide a new therapeutic approach for the treatment of type 2 diabetes.⁴³

11 β -HYDROXYSTEROID DEHYDROGENASE TYPE-1INHIBITORS:

11 β -hydroxysteroid dehydrogenase Type-1 converts inactive cortisone into active cortisol, thereby amplifies intracellular glucocorticoid action. 11 β HSD1 activity is elevated in adipose tissue of obese rodents and

humans.^{44,45} INCB13739 is an oral and selective 11 β HSD1 inhibitor being developed to treat type 2 diabetes. The efficacy and safety of the 11 β HSD1 inhibitor INCB13739 were assessed when added to ongoing metformin monotherapy in patients with type 2 diabetes exhibiting inadequate glycemic control (A1C 7–11%). After 12 weeks, 200 mg of INCB13739 resulted in significant reductions in A1C (-0.6%), fasting plasma glucose (-24 mg/dl), and homeostasis model assessment–insulin resistance (HOMA-IR) (-24%) compared with placebo. Total cholesterol, LDL cholesterol, and triglycerides were all significantly decreased in hyperlipidemic patients. Body weight decreased relative to placebo after INCB13739 therapy. 11 β HSD1 inhibition offers a new potential approach to control glucose and cardiovascular risk factors in type 2 diabetes.⁴⁶

C-JUN-N-TERMINAL PROTEIN KINASES (JNK) INHIBITORS: A NEW TARGET IN DIABETES TREATMENT:

Mitogen activated protein kinase (MAPK) is one of the major signalling pathways that transduces the extracellular signals into the cells. In mammals there are 3 subfamilies of MAPK identified till now. C-Jun-N-Terminal protein kinases (JNK)/ Stress activated protein kinases (SAPK), p38 MAPK and ERK. JNK is a multifunctional kinase involved in many physiological and pathological processes. The JNK pathway plays a major role in apoptosis where in it diminishes the anti-apoptotic activity and promotes the pro-apoptotic activity. JNK plays important role in many diseases like neurological disorders, diabetes, cancer, stroke, heart diseases and inflammatory disorders.⁴⁷ In diabetes, oxidative stress and endoplasmic reticulum stress is induced in various tissues, leading to activation of JNK pathway. JNK plays a central role in pathogenesis of diabetes and obesity.⁴⁸ In obese animals, JNK-1 gene knockout provides protection against insulin resistance and defective insulin receptor signalling. JNK interactive protein-1 (JIP1) knockout mice also shows reduced activity of JNK and increased insulin sensitivity.⁴⁹

JNK also modulates the function and survival of islet cells. JNK is involved in islet cell inflammation and death mediated by cytokines,⁵⁰ β -cell dysfunction⁵¹ and defective insulin production and finally inhibition of JNK improves glucose stimulated insulin secretion and reduced peripheral insulin resistance.⁵² From the above explanation it can be said that JNK plays a central role in pathogenesis of Type 2 DM and serves as a potential target for diabetes therapy.

Accordingly, there are 3 types of inhibitors namely ATP-competitive inhibitors, inhibitors to the substrate binding site and inhibitors of allosteric regulatory sites that target JNK.

FREE FATTY ACID RECEPTOR-1 (FFAR-1) AGONIST:

Also called G-protein coupled receptor-40, belongs to family of cell surface receptors. In humans, FFAR-1 are expressed in highest in pancreatic β cells. They are activated by unsaturated medium and long chain fatty acids including Linolenic acid. Their activation leads to production of second messengers namely Inositol triphosphate (IP3) and diacylglycerol (DAG). Activation of FFAR-1 leads to increased insulin secretion but only in the presence of glucose. The exact mechanism of glucose mediated insulin release by these agents is not clear, but the agonist targeting these receptors improve the glycemic control without the risk of concurrent hypoglycemia.⁵³

TAK-875

It is highly potent, orally active FFAR-1 agonist and is the first of its class to be tested as oral hypoglycemic agent. In a randomized, double blind, placebo-controlled and active comparator controlled phase 2 study, TAK 875 was compared with placebo and Glimepiride in patients who had not responded to diet or metformin treatment. It was a 12 week study where 426 patients were randomly assigned to TAK-875 6.25/25/50/100 or 200mg (n=303), placebo (n=61) and Glimepiride 4mg (n=62). At 12 weeks, significant reduction in HbA1c from baseline occurred in TAK-875 (ranging from -1.12% with 50mg to -0.65% with 6.25mg) and glimepiride (-1.05%) groups compared to placebo (-0.13%). Hypoglycemic events were similar in TAK-875 and placebo groups (2% and 3% respectively) and higher in glimepiride group (19%). Thus, overall treatment emergent adverse events were similar in TAK-875 and placebo group and were lower than in glimepiride group. Currently undergoing phase III trial.⁵³

CONCLUSION:

Diabetes mellitus is a disorder that currently has no cure, long term treatment is required to keep the blood sugar under control and to reduce the vascular complications. Effective and well tolerated orally administered drugs are given in the initial stages along with non-pharmacological treatment like dietary and life style modifications which manage to reduce the HbA1c by 1.5% to 2%. Metformin is likely to remain a well established first-line pharmacological treatment for patients with type 2 diabetes who are overweight because of its efficacy, long-term safety, and cardio protective properties.

But currently, diabetes is managed with combination therapy where several drugs are administered along with regular glucose monitoring and insulin is needed when there is significant beta cell failure or the patient is

not adequately responding to oral or injectable medications or when they are contraindicated. Even though none of the current drugs have any influence on disease progression the role of TZDs, DPP-4 inhibitors and incretin mimetics is questionable. Despite the FDA approved therapeutic options, many of the patients are not achieving adequate glycemic control. Newer drugs with novel mechanism of action and minimal risk of hypoglycemia, weight gain and preserving β cell mass are in various stages of development and have shown promising results. Further exploration of these targets will hopefully lead to safe and efficacious drugs that will become the mainstay of next generation therapeutics for diabetes. If they become successful, they may help in improving the clinical outcomes and reducing the clinical and cost burden of the disease.

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REFERENCES:

1. Powers AC. Diabetes Mellitus. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine. 18th ed. New York: Mc Graw Hill; 2012. p. 2968-3002.
2. Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States [Internet]. 2011[cited 2013 May]. Available from: http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed July 30, 2011.
3. Piya MK, Tahrani AA, Barnett AH. Emerging Treatment Options for Type 2 Diabetes. Br J Clin Pharmacol. 2010 Aug 4;70(5):631-44.
4. Houze JB, Zhu L, Sun Y, Akerman M, Qiu W, Zhang AJ, et al. AMG 837: a potent, orally bioavailable GPR40 agonist. Bioorg Med Chem Lett. 2012 Jan 15;22(2):1267-70.
5. Indian Council of Medical Research. ICMR guidelines for management of Type 2 Diabetes-2005 (section 1). 2005:1-4.
6. Ahrén B. GLP-1 for type 2 diabetes. Exp Cell Res. 2011 May 15;317(9):1239-45.
7. FDA. Exenatide FDA [internet]. 2009 [cited 2013 May]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021773s9s11s18s22s251bl.pdf

8. Gerich JE, Meyer C, Woerle HJ, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care*. 2001 Feb;24(2):382-91.
9. Wright EM, Hirayama BA, Loo DF. Active sugar transport in health and disease. *J Intern Med*. 2007 Jan;261(1):32-43.
10. Bakris GL, Fonseca VA, Sharma K, Wright EM. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. *Kidney Int*. 2009 Jun;75(12):1272-7.
11. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes*. 2005 Dec;54(12):3427-34.
12. Komoroski B, Vachharajani N, Boulton D, Kornhauser D, Gerald M, Li L, et al. Dapagliflozin, a novel SGLT2 inhibitor, induces dose-dependent glucosuria in healthy subjects. *Clin Pharmacol Ther*. 2009 May;85(5):520-6.
13. Bailey CJ, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomized, double-blind, placebo-controlled trial. *Lancet*. 2010 Jun 26;375(9733):2223-33.
14. Strojek K, Yoon KH, Hruby V, Elze M, Langkilde AM, Parikh S. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with glimepiride: a randomized, 24-week, double-blind, placebo-controlled trial. *Diabetes Obes Metab*. 2011 Oct;13(10):928-38.
15. Wilding JP, Norwood P, T'joen C, Bastien A, List JF, Fiedorek FT. A study of dapagliflozin in patients with type 2 diabetes receiving high doses of insulin plus insulin sensitizers: applicability of a novel insulin-independent treatment. *Diabetes Care*. 2009 Sep;32(9):1656-62.
16. List JF, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. *Diabetes Care*. 2009 Apr;32(4):650-7.
17. Ghosh RK, Ghosh SM, Chawla S, Jasdanwala SA. SGLT2 inhibitors: a new emerging therapeutic class in the treatment of type 2 diabetes mellitus. *J Clin Pharmacol*. 2012 Apr;52(4):457-63.
18. Phase 3 Results Show Canagliflozin as Add-On Therapy to Metformin and Pioglitazone Significantly Lowers Blood Sugar Levels in Adult Patients with Type 2 Diabetes [Internet]. 2012 Nov [cited 2013 May]. Available from: <http://www.prnewswire.com/news-releases/phase-3-results-show-canagliflozin-as-add-on-therapy-to-metformin-and-pioglitazone-significantly-lowers-blood-sugar-levels-in-adult-patients-with-type-2-diabetes-178048581.html>.
19. Bahare RS, Gupta G, Malik S, Sharma N. New Emerging Targets for Type-2 Diabetes. *Int.J.PharmTech Res*. 2011;3(2):809-18.
20. Levien TL, Baker DE. New Drugs in Development for the Treatment of Diabetes. *Diabetes Spectr*. 2009;22(2):92-106.
21. Ferré P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes*. 2004 Feb;53 Suppl 1:S43-50.
22. Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. *Pharmacol Res*. 2005 Feb;51(2):85-94.
23. Henry RR, Lincoff AM, Mudaliar S, Rabbia M, Chognot C, Herz M. Effect of the dual peroxisome proliferator-activated receptor-alpha/gamma agonist aleglitazar on risk of cardiovascular disease in patients with type 2 diabetes (SYNCHRONY): a phase II, randomised, dose-ranging study. *Lancet*. 2009 Jul 11;374(9684):126-35.
24. Metabasis Therapeutics Announces CS-917 Phase 2b Clinical Trial Results and Reports on Status of Pradefovir. [Internet]. 2007 Jul [cited 2013 May]:[about 1p]. Available from: http://www.drugs.com/clinical_trials/metabasis-therapeutics-announces-cs-917-phase-2b-clinical-trial-results-reports-status-pradefovir-1595.html
25. Nguyen QT, Thomas KT, Lyons KB, Nguyen LD, Plodkowski RA. Current therapies and emerging drugs in the pipeline for Type 2 Diabetes. *Am Health Drug Benefits*. 2011;4(5):303-11.
26. McCormack JG, Westergaard N, Kristiansen M, Brand CL, Lau J. Pharmacological approaches to inhibit endogenous glucose production as a means of anti-diabetic therapy. *Curr Pharm Des*. 2001 Sep;7(14):1451-74.
27. Martin WH, Hoover DJ, Armento SJ, Stock IA, McPherson RK, Danley DE, et al. Discovery of a human liver glycogen phosphorylase inhibitor that lowers blood glucose in vivo. *Proc Natl Acad Sci U S A*. 1998 Feb 17;95(4):1776-81.
28. Oikonomakos NG, Skamnaki VT, Tsitsanou KE, Gavalas NG, Johnson LN. A new allosteric site in glycogen phosphorylase b as a target for drug interactions. *Structure*. 2000 Jun 15;8(6):575-84.
29. Ahrén B. GLP-1 for type 2 diabetes. *Exp Cell Res*. 2011 May 15;317(9):1239-45.
30. Matschinsky FM, Magnuson MA, Zelent D, Jetton TL, Doliba N, Han Y, et al. The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy. *Diabetes*. 2006 Jan;55(1):1-12.

31. Veiga-da-Cunha M, Van Schaftingen E. Identification of fructose 6-phosphate- and fructose 1-phosphate-binding residues in the regulatory protein of glucokinase. *J Biol Chem.* 2002 Mar 8;277(10):8466-73.
32. Matchinsky FM, Zelente B, Doliba N, et al. Glucokinase activators for diabetes therapy. *Diabetes care.* 2011;34(suppl 2):S236-S43.
33. Madiraju SR, Poitout V. G protein-coupled receptors and insulin secretion: 119 and counting. *Endocrinology.* 2007 Jun;148(6):2598-600.
34. Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, et al. A role for intestinal endocrine cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release. *Endocrinology.* 2008 May;149(5):2038-47.
35. Chu ZL, Jones RM, He H, Carroll C, Gutierrez V, Lucman A, et al. A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology.* 2007 Jun;148(6):2601-9.
36. Yoshida S, Tanaka H, Oshima H, Yamazaki T, Yonetoku Y, Ohishi T, et al. AS1907417, a novel GPR119 agonist, as an insulinotropic and β -cell preservative agent for the treatment of type 2 diabetes. *Biochem Biophys Res Commun.* 2010 Oct 1;400(4):745-51.
37. Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A. Glycogen synthase kinase 3: more than a namesake. *Br J Pharmacol.* 2009 Mar;156(6):885-98.
38. Liu Z, Chai Q, Li YY, Shen Q, Ma LP, Zhang LN, et al. Discovery of novel PTP1B inhibitors with antihyperglycemic activity. *Acta Pharmacol Sin.* 2010 Aug;31(8):1005-12.
39. Sarath R, Kumar BR. Role of Leptin in Diabetes Mellitus. *Indian journal of fundamental and applied life sciences.* 2011 Apr;1(2):209-14.
40. Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab.* 2002 Jan;282(1):E18-23.
41. Buhl ES, Jessen N, Schmitz O, Pedersen SB, Pedersen O, Holman GD, et al. Chronic treatment with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. *Diabetes.* 2001 Jan;50(1):12-7.
42. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest.* 2001 Oct;108(8):1167-74.
43. Jacobson PB, von Geldern TW, Ohman L, Osterland M, Wang J, Zinker B, et al. Hepatic glucocorticoid receptor antagonism is sufficient to reduce elevated hepatic glucose output and improve glucose control in animal models of type 2 diabetes. *J Pharmacol Exp Ther.* 2005 Jul;314(1):191-200.
44. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes.* 2005;54 (Suppl. 2):S73-8.
45. Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, et al. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol.* 1993 Oct;14(4):303-47.
46. Wamil M, Seckl JR. Inhibition of 11 β -hydroxysteroid dehydrogenase type 1 as a promising therapeutic target. *Drug Discov Today.* 2007;12:504-20.
47. Cui J, Zhang M, Zhang YQ, Xu ZH. JNK pathway: diseases and therapeutic potential. *Acta Pharmacol Sin.* 2007 May; 28(5):601-8.
48. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem.* 2000 Mar 24;275(12):9047-54.
49. Waeber G, Delplanque J, Bonny C, Mooser V, Steinmann M, Widmann C, et al. The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. *Nat Genet.* 2000 Mar;24(3):291-5.
50. Abdelli S, Ansite J, Roduit R, Borsello T, Matsumoto I, Sawada T, et al. Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. *Diabetes.* 2004 Nov; 53(11):2815-23.
51. Bennett BL, Satoh Y, Lewis AJ. JNK: a new therapeutic target for diabetes. *Curr Opin Pharmacol.* 2003 Aug;3(4):420-5.
52. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, et al. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci U S A.* 2001 Nov 20;98(24):13681-6.
53. Kaku K, Araki T, Yoshinaka R. Randomized, double-blind, dose-ranging study of TAK-875, a novel GPR40 agonist, in Japanese patients with inadequately controlled type 2 diabetes. *Diabetes Care.* 2013 Feb; 36(2):245-50.