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REVIEW ARTICLE

PHARMACOINFORMATICS

"PROPOSING NEW TARGETS FOR THE TREATMENT OF DIABETES MELLITUS"

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ABSTRACT

Diabetes mellitus (DM) is considered a major public health issue because of its increasing prevalence and high morbidity and mortality. Recent research has proposed new targets for fighting against diabetes. The paradox of a well known receptor PPAR γ has been resolved by a scientist and he has proposed a new way to target this receptor. Further other new potential targets have been summarized in order to present a new dimension in the treatment of diabetes mellitus.

KEY WORDS

Diabetes mellitus , pparγ, Target, leptin

INTRODUCTION

Diabetes mellitus (DM) is considered a major public health issue because of its increasing prevalence and high morbidity and mortality. Type 2 diabetes develops as a result of complex multifactorial process with both lifestyle and genetic origins. Being a chronic non-communicable disease it requires a health-care team and family to participate in the multiple tasks in management.

Type 2 diabetes usually develops gradually and different stages can be identified (Eriksson et al 1989; Hamman 1992; Tuomilehto et al 1997). When genetically predisposed individuals become insulin resistant due to environmental exposures such as obesity or physical inactivity, they may develop post-prandial hyperglycaemia which is also called impaired glucose tolerance (IGT). Finally, when beta cell capacity is not sufficient to compensate for insulin resistance, hyperglycaemia worsens and overt diabetes will develop. It has been estimated that at the time of diagnosis of clinical type 2 diabetes only 50-60% of the pancreatic beta-cell capacity is left, due to the fact that the disease process has already existed for more than 10 years (UKPDS Group 1995). Therefore, the optimal strategy to reduce the increased burden of type 2 diabetes is the primary prevention of the disease, i.e. to tackle the worsening of glucose intolerance before harmful effects of hyperglycaemia will become permanent [1].

1)-A NEW WAY TO TARGET pparγ

INTRODUCTION TO THE STUDY:

Researchers at the Dana-Farber Cancer Institute and Scripps Florida have found an alternative mechanism by which peroxisome proliferation–activated receptor-γ agonists exert their antidiabetic effects [2]. The findings offer a reason for drug companies to take a fresh look at antidiabetic compounds that may have been previously dismissed due to their reduced agonistic activity but now may be seen to offer therapeutic benefits with the potential for fewer side effects than marketed peroxisome proliferation-activated receptor-γ (PPARG; PPARγ) agonists.

Indeed, the alternative mechanism may explain how partial PPARγ agonists in clinical development, including InteKrin Therapeutics Inc.'s INT131, can exhibit potent antidiabetic effects similar to those of full agonists such as GlaxoSmithKline plc's Avandia rosiglitazone and Takeda Pharmaceutical Co. Ltd.'s Actos pioglitazone. Avandia and Actos are marketed to treat type 2 diabetes and had combined worldwide sales of over $4.5 billion in 2009. However, patients taking these thiazolidinedione (TZD) drugs can experience side effects such as weight gain and edema. An FDA advisory panel recently split on whether Avandia should be taken off the market due to a potential increase in the risk of cardiovascular events [3]. Patents on Actos and Avandia will begin to expire in 2011 and 2012, respectively, placing further commercial pressure on companies targeting PPARγ. Now, a team led by Bruce Spiegelman, Korsmeyer professor of cell biology at Harvard Medical School and Dana-Farber, and Patrick Griffin, professor and chair of molecular therapeutics and director of the Translational Research Institute at Scripps Florida, has shown that PPARγ is phosphorylated by the obesity-activated cyclin dependent kinase 5 (CDK5), which results in misregulation of a subset of genes that are important for insulin sensitivity.The researchers then went on to show that some PPARγ agonists can block this CDK5-mediated phosphorylation (*see* **Table 1, "Targeting PPARγ"**).

Table1
Targetting PPARγ

Company	Product	Compound class	Lead status
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	Avandia rosiglitazone	Thiazolidinedione (TZD)	Marketed
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502)/Eli Lilly and Co. (NYSE:LLY)/ Pfizer Inc. (NYSE:PFE)	Actos pioglitazone	TZD	Marketed
Dr. Reddy's Laboratories Ltd. (NYSE:RDY)/Nordic Bioscience Holding	Balaglitazone (DRF 2593)	TZD	Phase III
InteKrin Therapeutics Inc.	INT131	Non-TZD	Phase IIb completed
Metabolex Inc.	MBX-102	Non-TZD	Not applicable[A]

[A]No longer in development for diabetes.

Critically, the extent to which a compound agonizes PPARγ is not correlated with the extent to which it inhibits phosphorylation, suggesting that these compounds have two distinct and separable activities.

A NEW WAY TO TARGET PPARγ

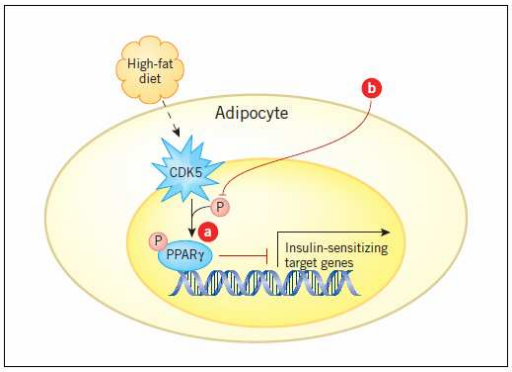

Figure1

A new way to target PPARγ. Harvard Medical School researchers suggest that compounds that block phosphorylation of peroxisome proliferation–activated receptor-γ (PPARG; PPARγ) could provide a better way of treating obesity and type 2 diabetes than marketed thiazolidinediones (TZDs), which agonize the transcription factor, resulting in not only insulin sensitivity but also unwanted downstream effects. In adipocytes of mice fed a high-fat diet, cyclin dependent kinase 5 (CDK5)-dependent phosphorylation (P) of PPARγ at serine 273 is increased, which decreases downstream expression of insulin-sensitizing genes [a]. By blocking CDK5-dependent phosphorylation, PPARγ is able to increase the expression of these genes [b] [4].

PPARγ agonism has classically been measured as a compound's ability to stimulate transcription of a PPARγ-responsive reporter gene in cell culture. A compound was dubbed a partial agonist if it stimulated less reporter activity than Rosiglitazone, the standard benchmark**.** Spiegelman's team demonstrated the function of CDK5-mediated phosphorylation by transplanting fibroblasts subcutaneously into mice and allowing them to differentiate into adipocytes. Transplantation of adipocytes that expressed PPARγ lacking this phosphorylation site led to greater levels of

adiponectin, a key hormone that maintains insulin sensitivity, whereas transplantation of adipocytes that expressed wild-type PPARγ did not. In contrast, mutation of this phosphorylation site had no effect on the level of PPARγ agonism in classical transcriptional reporter assays, whether in the presence or absence of rosiglitazone. However the most striking result was shown in patients taking a TZD agonist, in this case Avandia, in which a decrease in PPARγ phosphorylation was significantly correlated with improvement in glucose infusion rate, a measure of insulin sensitivity.

Although all these compounds were known to interact with a large ligand-binding pocket on PPARγ, Griffin's laboratory used hydrogen-deuterium exchange mass spectroscopy (HDX-MS) to show that they cause distinct conformational changes in the receptor that could influence subsequent phosphorylation. Griffin stated that partial agonists altered the conformational dynamics of specific regions of PPARγ differently from full agonists and indicated that they might affect coactivator recruitment by generating a new interaction surface or by altering post-translational modifications. These details are important because they provide a mechanistic explanation for how non-TZDs still have potent antidiabetic effects even though they only partially agonize the PPARγ receptor. These types of compounds are often referred to as selective PPAR modulators (SPPARMs) and include **Metabolex Inc.**'s MBX-102 and InteKrin's INT131. SPPARMs such as INT131 have been characterized that achieve separation of side effects from antidiabetic activity. It is not known yet whether INT131 blocks this phosphorylation event. InteKrin has completed a Phase IIb trial of INT131 and plans to move forward with Phase III testing in type 2 diabetes, although it hasn't provided a timeline. INT131 is the SPPARM furthest along in development [4].

Pparanoia

Although companies could re-examine compounds that bind PPARγ but were dismissed due to a lack of agonism in classical transcriptional reporter assays, the stigma attached to PPARγ as a therapeutic target still may dissuade further investigation.

Companies have run away from PPARγ, and it will be interesting to see if this reignites their interest. As a author of this review I believe the stigma surrounding PPARγ is unwarranted; it is a validated target with a big market, and it is surprising that many companies have completely left it behind. One problem is that no one knows which PPARγ target genes cause the unwanted side effects. Until we know why patients taking Avandia have an increased rate of heart attack, companies will be reticent to target PPARγ [4].

2)-leptin instead of insulin

Introduction to the study

Texas researchers have shown that the fat-derived hormone leptin could substitute for insulin to control blood sugar fluctuations in patients with type 1 diabetes.1 the work has caught the attention of ***amylin pharmaceuticals inc.,*** which is now collaborating with the researchers to set up a Phase II trial of this new therapeutic approach. Ordinarily, insulin secreted by pancreatic islet β cells after a meal prompts liver and muscle cells to convert excess blood sugar to glycogen. In type 1 diabetes, loss of these β cells from autoimmune attack leaves patients without a way to control high blood sugar; over time, this leads to kidney and cardiovascular damage. Insulin replacement has been the standard of care for type 1 diabetes since the 1920s, when the hormone's role in regulating sugar uptake from the bloodstream was discovered. But inaccurate dosing of injected insulin can lead to dangerous fluctuations in blood sugar, making continuous blood sugar monitoring and regular high-dose insulin injections a necessity for diabetics. Roger Unger, professor of internal medicine at **The *University of Texas Southwestern Medical Center at Dallas, and his showed that in a mouse*** model of type 1 diabetes, the adipocyte-derived hormone leptin was as effective as insulin in controlling blood sugar

while eliciting fewer undesirable side effects. Leptin signaling in the brain had previously been implicated in appetite and weight control, but the effect of leptin on blood sugar has only recently been discovered [5].

Better control

Drawing from earlier studies with leptin transgenic mice that showed changes in glucose metabolism, Unger's team tested the effect of subcutaneous leptin in nonobese diabetic mice, a standard model for type1 diabetes. Compared with insulin-treated mice, leptin-treated mice showed similar reductions in blood sugar and similar metabolic fingerprints of hyperglycemia such as glucose and ketones in the urine. The team also found that combining low doses of leptin and insulin led to steadier long-term blood glucose control than high doses of insulin alone, suggesting that leptin could be used as an adjunct to conventional insulin therapy. Unger's team also found that leptin had a more benign effect on diet and lipid metabolism. Leptin-treated mice had less food intake and lower levels of fatty acids and cholesterol biosynthetic enzymes than insulin-treated controls. I, thus think that leptin could be useful for preventing dyslipidemia, a common side effect of long-term insulin treatment that leads to obesity and cardiovascular complications in many patients [5].

3)-FGF-21 a new target

FGF-21 is a liver-derived polypeptide that appears to have considerable potential for the treatment of diabetes mellitus [6, 7]. In a recent paper, FGF-21 was found to act as an adipocyte-specific inducer of glucose uptake and to lower plasma triglyceride (TG) levels over an extended period [7]. Notably, the effect is not immediate, and it is independent of insulin. FGF-21 effects on glucose uptake are additive, not synergistic with insulin. Moreover, unlike insulin, adipocyte responses to FGF-21 required exposure over a number of hours. The actual mechanism involved is unclear, but could involve a number of points along the glucose metabolic pathway (Figure 2).

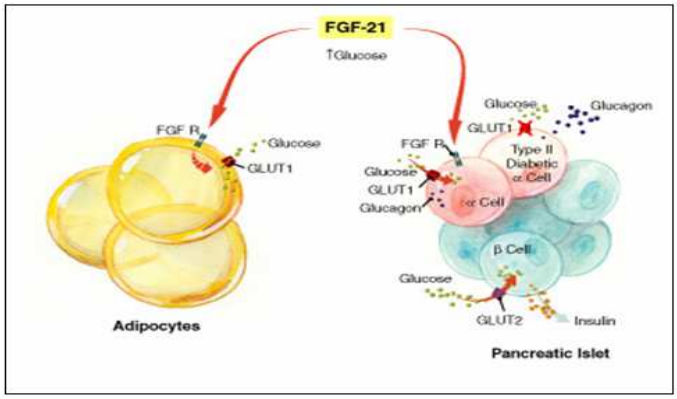

Figure 2

Potential targets for FGF-21-mediated glucose uptake: FGF-21 may stimulate glucose uptake into adipocytes via FGF R modulation of adipocyte GLUT1. In addition, FGF-21 may enhance glucose uptake into glucagon-secreting pancreatic a-cells. In type II diabetics, this could have the effect of increasing insulin sensitivity by suppressing glucagon release, decreasing circulating glucose, and lowering the amount of insulin production required by the pancreatic ß-cells [16].

Normally, dietary glucose is absorbed into the intestinal vasculature and quickly encounters ß-cells of the pancreatic islets. Rodent ß-cells express GLUT2, a member of the SLC2 family of glucose and polyol transporters [8,9]. GLUT2 is unusual in that it is constitutively expressed on the cell surface and allows almost free diffusion of its target,

glucose. Thus, any increase in extracellular glucose will be reflected by an almost immediate proportional increase in intracellular glucose. All rises in intracellular glucose are quickly followed by insulin release. The release is biphasic, peaking after three minutes, declining somewhat, and rising again after ten minutes for the duration of the glycemic episode [10]. Released insulin encounters insulin receptors expressed on the principal targets of insulin such as muscle and fat. The first wave of insulin activates plasma membrane GLUT4 receptors, opening channels for glucose influx. The second and continuing wave of insulin induces GLUT4 translocation from internal vesicles to the plasma membrane, increasing the influx of glucose.10 Insulin resistance is a hallmark of type II diabetes, and is characterized by an inability to efficiently transport glucose into muscle and (white) fat. Approximately 75-90% of dietary glucose goes into muscle fibers, while 10% of plasma glucose is taken up by adipocytes [10, 12]. GLUT4 is reportedly poorly expressed on muscle and fat in diabetes [9,11]. This reduction could lead to hyperglycemia, since the “funnel” for glucose deposition would be reduced. GLUT4 would seem to be a possible target for FGF-21, an agent that causes glucose uptake.

Although it is tempting to speculate that FGF-21 might exert its glucose uptake effects via GLUT4, this doesn’t appear to be the case. Remarkably enough, FGF-21 seems to impact another GLUT transporter, GLUT1. GLUT1 activity seems to be independent of insulin action (at least on monocytes), and it is reported to be the predominant GLUT on human ß-cells (in contrast to rodent) [8,13-15]. FGF-21 is hypothesized to impact GLUT1 on adipocytes, but not skeletal muscle [7]. The effect is probably indirect, as some isoform of FGF R1 and/or FGF R2 is likely to be the receptor for FGF-21 [7]. Although GLUT1 is a glucose transporter, it is unclear what effect FGF-21 could have on facilitated adipocyte glucose transport. Glucose entry into adipocytes generally results in its storage as TG. In the liver, plasma-derived glucose can be broken down to acetyl-CoA, and then reassembled from acetyl-CoA, two carbons at a time, into 16- and 18-carbon fatty acids. These can then be transported to the adipocyte via very low density lipoprotein (VLDL) where they are bound to glucose-derived, 3-carbon glycerol to form TG. In theory, this should result in increased TG stores and, by inference, enlarged adipocytes. However, FGF-21 transgenic mice, in which the human protein is over-expressed in the liver, exhibit, white adipocytes that are smaller than normal. If FGF-21 does facilitate glucose influx, perhaps it does so on an expanded white adipocyte mass. Alternatively, adipocyte glucose may be metabolized and not used for fat storage. FGF-21 has also been proposed to impact glucagon metabolism [16].

4)-EUKARYOTIC TRANSLATIONAL INTIATION FACTOR 5a

A North American team has identified a proinflammatory pathway involving eukaryotic translation initiation factor 5A, a poorly understood translational initiation factor, as a potential player in diabetes.1EIF5A undergoes a unique post-translational modification in which a single lysine residue is converted into an unconventional amino acid called hypusine that is found nowhere else in nature. This conversion requires the sequential activity of two enzymes: deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase/ monooxygenase (DOHH).

Although mouse knockouts have shown that Eif5a hypusination is not essential for survival under most circumstances, work published in 2008 suggested that Eif5a promoted lung inflammation and sepsis in mice.2 Because many proinflammatory pathways are at play in the pancreas in both type 1 and type 2 diabetes, the researchers hypothesized that EIF5A might also contribute to pancreatic inflammation. EIF5A mediates stress-induced inflammation in the pancreas, and it adds to growing evidence of an inflammatory component to both type 1 and type 2 diabetes that exacerbates islet β cell destruction.3. This pathway seems to be a potential target for fighting against inflammation in diabetes.

The team first showed in a mouse model of diabetes that siRNA knockdown of Eif5a reduced islet cell loss and improved glucose tolerance compared with what was seen using control siRNA or no treatment. EIF5A promoted inflammation by stimulating the translation of mRNA encoding inducible nitric oxide synthase (NOS2; iNOS), an intracellular effector of inflammation-associated cell death. EIF5A knockdown in mice and cultured human islet β cells reduced levels of iNOS compared with those seen using control siRNA or no treatment. The team then tested whether hypusination was required for EIF5A's effects on inflammation. The team found that a commercially available small molecule inhibitor of DHPS blocked the translation of iNOS, glucose response and insulin secretion in cultured β cells.

Diabetic mice treated with the DHPS inhibitor had lower iNos and inflammatory cytokine levels and better glucose tolerance and insulin secretion than untreated controls. To explain why blocking EIF5A hypusination should prevent both production of inflammatory cytokines and iNOS translation, Mirmira suggested that the pathway could be active in a variety of cell types.

"EIF5A is active in both islet cells and inflammatory cells," said Mirmira. "There is signaling through this pathway going on in the β cells, but there may be an additional benefit to inhibiting the process in the immune cells." Mirmira next plans to test the effect of both blocking EIF5A hypusination in other models of diabetes and combining hypusination-blocking compounds with
anti-inflammatory molecules that target other pathway [17].

CONCLUSION

In this article, I have tried to put forth new targets for fighting against the complications of diabetes. All the proposed targets are verified by the experiments done in the biotechnology and animal testing laboratories. So being the author I would suggest the researchers to focus on these potential targets.

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