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Review Article

Role of Glycogen Synthase Kinase (GSK-3) in Type-2 Diabetes and GSK-3 Inhibitors as Potential Anti-Diabetics

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Abstract

Glycogen Synthase Kinase (GSK-3) is a key enzyme involved in glycogen metabolism, protein synthesis, etc and overexpression of GSK-3 in skeletal muscle of humans is associated with impaired ability of insulin to activate glucose disposal leading to development of type-2 diabetes. Studies have demonstrated that selective and sensitive inhibition of GSK-3 causes improvements in insulin stimulated glucose transport activity. Identifying the binding sites and selectively targeting GSK-3 with GSK-3 inhibitors may emerge as a new strategy for the treatment of diabetes.

1. INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, negative nitrogen balance and ketonemia.[1] According to the Statistics Published by the International Diabetes Federation (IDF) (Belgium) & WHO, More than 371 million people in the world have diabetes and the number of people with diabetes is increasing in every country. Considering the Indian population, over 63 million have now been diagnosed with diabetes in India (2012). 4.8 million people have died and USD 471 billion was spent due to diabetes in 2012 worldwide. And the most disturbing statistic is that more than half the people who die of diabetes are under the age of 60. These statistics are clearly discouraging. Diabetes in the near future will be a major cause of mortalities all over the world. It must be however understood that diabetes can be easily controlled with proper and timely medication. Current Pharmacological approaches are unsatisfactory in improving consequences of insulin resistance. There is no single approach to treat this disease and usually a combination therapy is adopted to treat this disease.[2]

Skeletal muscle insulin resistance and accompanying hyperinsulanemia play a critical role in Type 2 (NIDDM) diabetes.[3] Additionally, increase in hepatic glucose production aggravates the state of glucose dysregulation.[4] Therefore intervention that can ameliorate insulin resistance and hepatic glucose production helps to reduce conversion rates from conditions of impaired tolerance and Pre-diabetic to overt Type 2 diabetes. [5, 6] The etiology of skeletal muscle insulin resistance is multifactorial and involves defective expression and functionality of multiple elements in insulin signaling cascade that regulates glucose transport process. Evidence to date indicates the role of Serine/threonine protein kinase Glycogen Synthase Kinase (GSK-3) which mediates addition of phosphate molecule to serine/threonine amino acid residues in insulin resistance.[7] The expression and activity of GSK-3 plays a critical role in diverse cellular processes such as glycogen synthesis, glucose transport, protein synthesis, gene transcription, cell differentiation, intracellular signaling pathways regulated by insulin and in muscle contractile activity.[8-10] The present review will focus on the action of GSK-3 in mediating glucose transport and its role in insulin resistance. Subsequently we will also stress upon GSK-3 inhibitors and their role in treating Type-2 diabetes.

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2. GSK-3 IN DIABETES AND INSULIN RESISTANCE

Glycogen Synthase Kinase 3 (GSK-3) is a serine/Threonine protein kinase that mediates the addition of phosphate molecules on to serine and threonine amino acid residues. It consists of two isoforms α and β [11,12,13,14]. It was one of the first kinases to be identified and studied initially in its regulation of glycogen synthase. However now the interest in GSK-3 has gone far beyond glycogen metabolism and is known to occupy a central stage in many cellular and physiological events. In humans two genes which map the two distinct but closely related forms of GSK-3 are GSK-3α and GSK-3β. GSK-3 has recently been a subject of much research because it has been implicated in a number of diseases like Type 2 Diabetes Mellitus, Alzheimer's disease, Cancer, Bipolar disorder, etc.

GSK-3 is constitutively active in cells, and can be acutely inactivated by insulin signalling through the sequential activation of IRS-1, PI3-kinase, and ultimately via the action of Akt to phosphorylate specific serine residues on the enzyme.[15] The very similar time courses for the insulin-dependent activation of Akt and inactivation of GSK-3 is consistent with the concept that GSK-3 is a physiologically relevant substrate for Akt.[16] An additional substrate of GSK-3 is IRS-1, and phosphorylation of IRS-1 on serine and threonine residues leads to impairment of insulin signaling.[17] These observations support the hypothesis that GSK-3 can serve as a negative modulator of insulin action on glycogen synthase and, potentially on glucose transport activity.

Skeletal muscle tissue consists of 40% of the body mass of humans and other mammalian species and it is the major tissue responsible for disposal and storage of glucose in response to a stimulus initiated by insulin or glucose in blood. The glucose

transport activity is regulated by insulin by activation of a series of different intracellular proteins. An Insulin receptor on the cell has an α and a β subunit. Insulin binding to the α subunit of the receptor stimulates the tyrosine kinase activity of the membrane spanning β subunits which leads to autophosphorylation of these subunits which enhances the tyrosine kinase activity. Different substrates are associated with the glucose uptake pathway namely PI3-kinase (Phosphoinositol-3-kinase) and IRS-1 (Insulin receptor substrate). Once autophosphorylation of insulin receptors takes place it activates the further cascade involving IRS-1, PI3-Kinase and Akt. This is ultimately responsible for translocation of Glucose inside the cell through GLUT-4 transporters. Experimental Evidences support the fact that GSK-3 is responsible for phosphorylation of the above mentioned substrates and is associated with insulin resistance and hyperglycemia.

3.1. Experimental Evidence

GSK-3 is elevated in tissues of insulin-resistant obese rodent models, including high fat-fed mice[18], obese Zucker rats [19], and the Zucker Diabetic Fatty (ZDF) rat, a model of type 2 diabetes.[20] In addition, GSK-3 is enhanced in skeletal muscle of obese humans [19] and type 2 diabetic humans.[21]

The elevation in GSK-3 protein in skeletal muscle of type 2 diabetic subjects is negatively correlated with both insulin-stimulated skeletal muscle glycogen synthase activity and whole-body glucose disposal.[21] Collectively, these findings are consistent with GSK-3 being a molecule that can indirectly modulate glucose transport and metabolism in skeletal muscle. An important finding regarding the molecular basis of insulin resistance is the observation that serine phosphorylation of IRS-1 near the phosphotyrosine binding site (Ser 307 in rat IRS-1) is associated with an impairment of tyrosine phosphorylation of IRS-1 by the insulin receptor and with a decrease in PI3-kinase activity. [22] As GSK-3 is known to phosphorylate IRS-1 on serine residues,[18] the possibility exists that GSK-3 mediates it effect to decrease insulin signaling in skeletal muscle by phosphorylating Ser 307 or another critical serine residue on IRS-1. Interestingly, recent evidence indicates that GSK-3 can phosphorylate Ser 332 on IRS-1 *in- vitro*, an effect that is reduced by the non-specific GSK-3 inhibitor lithium.[23]

Moreover, mutation of this critical serine residue on IRS-1 in Chinese hamster ovary cells overexpressing insulin receptors increases IRS-1 tyrosine phosphorylation and enhances insulin activation of Akt.[23] The effect of GSK-3 overexpression on whole-body glucose tolerance in mice has been recently investigated.[24] These investigators produced a mouse model that selectively overexpresses the GSK-3ß isoform in skeletal muscle by 5-fold. Relative to nontransgenic control mice, male GSK-3ß transgenic mice are characterized by increased fat mass, decreased muscle protein expression of IRS-1, and decreased glycogen synthase activity and glycogen levels in muscle. Moreover, in response to an oral glucose challenge, the male GSK-3ß transgenic mice display an exaggerated glucose response and elevated insulin levels, indicating a decrease in whole-body insulin sensitivity. These male GSK-3ß transgenic mice also show evidence of dyslipidemia, as both fasting plasma levels of free fatty acids and triglycerides are elevated.[24] The metabolic effects of GSK-3 overexpression were also investigated by the use of constitutively active forms of GSK-3α and GSK-3ß that were “knocked” in mice.[25] In contrast to the effects of muscle-specific GSK-3ß overexpression,[24] the mice with constitutively active GSK-3α and/or GSK-3ß displayed normal growth and no alterations in plasma insulin, muscle glycogen levels, glucose tolerance, and insulin-stimulated muscle glucose uptake[25].

GSK-3 can phosphorylate glycogen synthase on multiple serine residues and inactivate this enzyme, thereby reducing glycogenesis[26, 27]. This will indirectly potentiate hepatic glucose production derived from glycogenolysis. There is also indirect evidence for a role of GSK-3 in hepatic gluconeogenesis from *in-vitro* studies indicating that GSK-3 can phosphorylate (on Ser 129) and activate cAMP-responsive element binding (CREB) protein. [28] The activated CREB could then increase PEPCK expression [29] and thereby upregulate the rate of hepatic gluconeogenesis. However, selective *in-vitro* inhibition of GSK-3 can repress promoter activity of the PEPCK and G6Pase genes, [30] which could reduce the rate of gluconeogenesis.

3.2 Structural Biology

GSK-3β has been crystallized recently.[31,32] The overall shape is shared by all kinases, with a small N-terminal lobe, which consists of β-sheets and a large C-terminal lobe, which is formed essentially of α-helices.[33] The ATP- binding pocket is located between the two lobes; Arg 96, Arg 180 and Lys 205 form a small pocket where the phosphate group of the primed substrate and the pseudosubstrate bind. The two forms of GSK-3 display 84% overall identity with the main difference being an extra Gly-rich stretch in the N-terminal domain of GSK-3α. However, they are not interchangeable functionally, as demonstrated by the embryonic-lethal phenotype observed when the gene that encodes GSK-3β2, an alternative splicing variant of GSK-3β that contains a 13-amino acid insertion in the catalytic domain, has been identified.

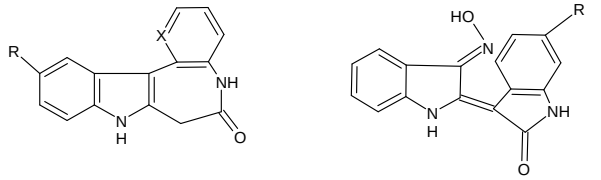
GSK-3 is regulated at multiple levels. First GSK-3β is regulated by post translational phosphorylation of Ser 9 (inhibitory) and Tyr 216 (activating) (Ser 21 and Tyr 279, respectively in GSK-3α). Phosphorylated Ser9 in the N-terminal domain of GSK-3β acts as a pseudo-substrate that blocks the access of substrates to the catalytic site, and phosphorylation releases this inhibition. Secondly, GSK-3β is regulated by interactions with many other proteins. Presenilin and Axin act as docking proteins that allow the substrates to make contact with the priming kinase and GSK-3. Third GSK-3 action requires the priming phosphorylation of its substrate by another kinase on a serine residue located four amino acids C-terminal to the GSK-3 phosphorylation site. Fourth, GSK-3 is regulated through its intracellular distribution. [34]

GSK-3 functions by phosphorylating a serine or threonine residue on its target substrate. A positively charged pocket adjacent to the active site binds a "priming" phosphate group attached to a serine or threonine four residues C-terminal of the target phosphorylation site. The active site, at residues 181, 200, 97, and 85, binds the terminal phosphate of ATP and transfers it to the target location on the substrate.

3. GSK-3β INHIBITORS

Selectivity is the major issue when GSK 3 inhibitors are used as pharmacological tools to demonstrate the involvement of GSK-3 in cellular processes. By contrast absolute selectivity is not necessarily the best approach when considering GSK-3 inhibitors as potential treatment of Type-2 Diabetes. This is because high selectivity may lead to development of resistance.[35] Because the ATP binding pockets of GSK-3α, GSK-3β are similar, inhibitors that target these sites are unlikely to differentiate between the two isoforms.

The selectivity of many GSK-3 inhibitors is poorly known, based on their evaluation with limited panels of kinases. [36, 37] Paullones, especially kenpaullone, 6-bromosubstituted indirubins [38, 39, 40] appear to be among the most selective inhibitors. (Fig. 1)



R = H; X = C Paullone

R = Br ; X = C Kenpaullone

R = 6-Br-substituted-Indirubin

Fig. 1: Structures of paullones, kenpaullone and indirubins

Promising results were obtained with maleimide based two structural analogues viz, arylindolemaleimide SB216763 and anilinomaleimide SB415286. They were found to inhibit both the isoforms of GSKs in ATP-competitive manner. SB216763 was found to activate glycogen synthase activity and glucose incorporation into glycogen. This action is additive to its ability to elicit insulin secretion.[41] SB415286 was not found to enhance basal glucose transport in insulin responsive cells like L6 myotubes. [42]

The compounds CT98014, CT98023, CT99021 which are substituted aminopyrimidines were found to activate glycogen synthase activity in nanomolar range in cell lines (Fig.2).[42]

Pyrazole based GSK-3 inhibitors were also developed. It was found that Compound 1 (Fig. 3) interacts with β-strands of GSK-3 by hydrogen bond interactions; in addition hydrazone portion of compound was found to adopt S-Cis conformation while binding to

ATP-binding pockets of GSK-3. Phenyl ring at 1 position should be present in coplanar conformation with pyrazolopyrimidine. In an attempt to maintain S-Cis coplanar conformation of molecule several other analogues 2-6 were synthesized. Compound 2 and 6 were found to be equally potent when compared with 1. Inactivity of compound 5 was attributed to interaction occurring in between imidazole 3N and hydrazone N in its vicinity which ultimately affects cis conformation of molecule. Conformations of phenyl ring, Steric interactions occurring between Phenyl- H and 2-H as in case of compound 4 and loss of hydrogen bonding interaction due to methylation in case of compound 3 is attributed to loss of activity of these compounds.[43] Molecular modeling studies of novel 8-arylated purines also enlighten our understanding of GSK-3 binding sites. It was found out that 8-arylated compound with smaller (Fig.4) hydrogen bonding substituents like CN, Cl, OH, NH inhibit GSK-3 and CDK-5 in dose dependent manner, whereas compounds with bulkier substituents like propyloxy, bromo,3-hydroxypropylamino have no activity. This was due to coplanarity that exists between three aromatic rings of compounds with adenine group of the enzyme. Aromatic ring at 8th position is found embedded in adenine pocket whereas substituents on position 6 occupy the place of phosphate. In case of Set II purine core and its 8-aromatic substituents with bulky groups occupies the same region on enzyme GSK-3 as in case of set I but purine core and phenyl ring are non-coplanar with each other. Presence of amide carbonyl group is found to provide an additional hydrogen bonding interaction with Arg-141 of enzyme. In addition to this, hydrophobic interaction between 8-substitued aromatics having smaller groups and protein adenine pocket of enzyme, electrostatic interactions with N3 of purine and Arg 141 and interaction between –NH-9 and -CO of Ile 62 are responsible for stabilization and proper fit of compounds which is not observed in case of bulkier substituents as they are found to rotate the purine ring by angle of 160˚ from the phenyl ring. The difference observed in activity of set I and set II compounds with bulkier substituents are also attributed to structural differences observed with G-loop of β-sheet of GSK-3 and CDK-5. Phe-36, Leu-88 of GSK-3 are found to establish hydrogen bonding interactions whereas in case of CDK-5, Tyr-15 and Arg-36 get involved in such interactions.[44]

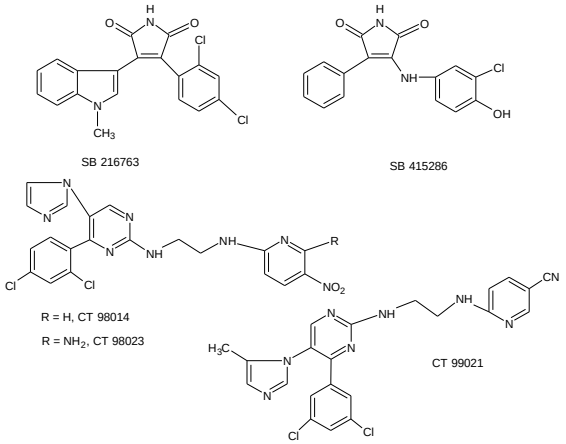


Fig. 2: Structures of maleimide based and aminopyrimidine based GSK-3 inhibitors

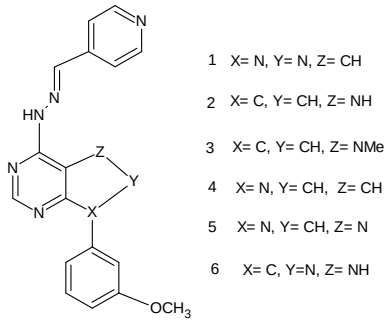


Fig. 3: Pyrazole based GSK-3 inhibitors

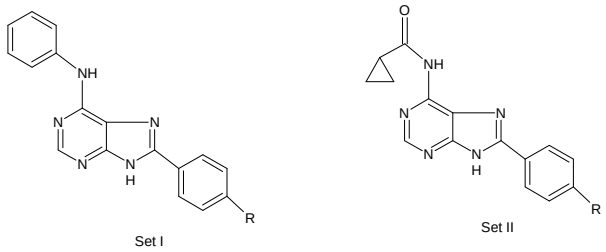


Fig. 4: 8-arylated purines as GSK3 inhibitors

Another report on 7-hydroxybenzimidazoles and its derivatives also provides greater insight on interaction that may occur while binding of the compounds with active sites of GSK-3. (Fig.5) In this series, 7-hydroxy group of benzimidazole functions as both hydrogen bond donor and acceptor with Asp 113 and Val 135 of catalytic domain of GSK-3. As 7-hydroxylated compounds were found to be more metabolically prone, they are further modified. The resulting imidazopyridine analogues were found to be equally potent inhibitors of GSK-3. This suggests that pyridinyl nitrogen is behaving as hydrogen bond donor and acceptor instead of phenolic –OH in latter case and one such group should be present in an inhibitor which is capable of interacting with Asp 113 and Val 135 of catalytic domain. Substituents at 2 positions were found to be responsible for pharmacokinetic behavior of compounds. [45]

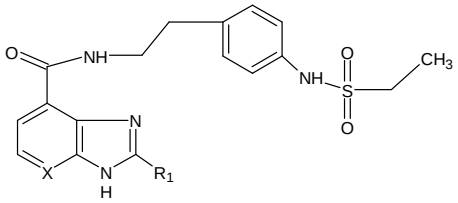


Fig. 5: 7-hydroxybenzimidazole and synthetic analogues as GSK-3 inhibitors

4. CONCLUSION

We are only beginning to understand the molecular mechanism underlying development of insulin resistance and function of GSK-3β enzyme. It will be important in future investigations to obtain more thorough assessment of how these GSK3 inhibitors might beneficially impact the insulin signaling pathway in skeletal muscle. Identifying the binding sites and subsequent development of inhibitors is a novel approach for treatment for type-2 diabetes.

As GSK-3 inhibition may also induce β-catenin regulated transcription factor, leading to development of cancer, it is an area of major concern with chronic inhibition of GSK3. Current research on GSK-3 inhibition does not support this issue of increasing cancer risk in animal models. Hence, this critical issue needs to be addressed with detailed study in near future. The information gained from acute and chronic inhibition of GSK-3β based on animal and cell line studies will be of great importance in guiding future investigations, to improve insulin sensitivity and use of GSK-3β inhibitors as antidiabetics.

5. ACKNOWLEDGEMENT

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