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Original Article

Natural Aldose Reductase Inhibitors Act as Potent Agonists of PPARγ

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ABSTRACT

Background: Diabetes and cancer have been the leading cause of mortality all over the world. Studies on association between diabetes and cancers over a decade indicate a positive relationship between them. Epidemiologic evidence suggests that people with diabetes are prone to various types of cancers. This work suggests a novel strategy for the treatment of diabetes and cancers. **Methods:** Extra-precision (XP) docking strategy, was used to predict the binding interactions of partial, full agonists of PPARγ and aldose reductase inhibitors on the PPARγ crystal structures. Binding interactions of PPARγ with the partial agonists, NTzDpa and MEKT76 and the full agonist Rosiglytasone were exploited to identify partial and full agonists of PPARγ among aldose reductase inhibitors. **Results:** Full and partial agonists of PPARγ inhibit various cancers, by suppressing the factors associated with neovascularisation. Partial agonists of PPARγ are prefered than full agonists like thiazolidinediones, reported to have serious side effects. Aldose reductase inhibitors used to treat diabetic complications, show binding interactions similar to the agonists of PPARγ and could hold a heuristic approach in treating diabetes and cancers. **Conclusion:** Aldose reductase inhibitors, tetrahydrocurcumin, catechin-5-O-gallate, rutin, (2S)-2'- methoxykurarinone, epalrestat, 8-lavandulylkaempferol depict interactions of partial agonists of PPARγ and could be further studied for their dual role as agonist and antagonist of the proteins PPARγ and Aldose reductase respectively.

Key words: Aldose reductase inhibitors, Cancer treatment, Diabetes, Natural compounds, PPARγ agonists.

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INTRODUCTION

Diabetes and cancer are the leading causes of mortality all over the world. The prevalence of both diabetes and cancer has increased worldwide despite the use of advanced treatment strategies.[1] Hyperglycemia induced oxidative stress and persistent inflammations are the major causes for cancerous tumors. Studies indicate the involvement of Aldose reductase (ALR2) activation in mediating the inflammatory signals induced by various factors. Furthermore, ALR2 was found to be involved in the activation of nuclear factor kappaB (NF-κB) by inducing the formation of advanced glycation end product (AGE) precursors that bind to the AGE receptors and influence the activation of the transcription factor. The activation of NF-κB and activator protein 1 (AP-1), initiate inflammatory response, since they transcribe genes involved in inflammation, ontogenesis' and apoptosis.[2] Cell proliferation in tumerogenesis is induced by the inflammatory cytokines and the growth factors. A clear understanding on the pathway involving ALR2 induced oxidative stress, oxidative stress induced inflammation and induction of cancer formation could be used to identify novel inhibitors of ALR2 for the treatment of diabetes and cancer.

Studies on cancers have shown that hypoglycemic drugs could control cancers, though the mechanism of correlation between diabetes and cancers are unclear.[3] Despite various research efforts made there is dearth of clinical data on diabetes linked cancers. Investigations on different antidiabetic drugs on different human cancer cell lines indicate that cancer proliferation is prompted by glucose and insulin that cause chemoresistance. Antidiabeteic drugs, Metformin and Rosiglitazone were reported to induce apoptosis and control cancer growth by affecting signaling in the protein kinases B (AKT)/mammalian target of rapamycin pathway[4] thereby proved to be used as an effective drug for treating type2 diabetes in cancer patients. Inhibition of ALR2 by Gedunin, a tetranortriterpenoid isolated from the neem tree, caused inactivation of the phosphatidyl inositol-3-kinase (PI3K)/Akt, and NF-κB that caused inhibition of angiogenesis.[5] Inhibition of ALR2 prevented Transforming growth factor *beta* (TGF-β) induced colon cancer by increasing the rate of program cell death through ROS/AMPK/mTOR pathway.[6] ALR2 inhibitor, Fidarestat is under phase III trials for Diabetic Neuropathy without any adverse effects, and has prevented the proliferation of human colorectal cancer (CRC) cells and also suppressed the expression of inflammatory cytokines and factors such as Cyclooxygenase-2 and prostaglandin E2.[7] This might be the reason behind the action of antidiabetic drugs on cancers.

In this work, we have identified another mechanism, linking diabetes and cancer through a different pathway, linking the action of fibrates. Fibrates (Fibric acid derivatives) are a class of amphipathic carboxylic acids that are prescribed for metabolic disorders mainly hypercholesterolemia. They have been used primarily in patients with type 2 diabetes and dyslipidemia. Fibrates were primarily used for the treatment of microvascular complications especially diabetic retinopathy. Chlorofibrate was the first fibrate drug to be used in 1960's for the treatment of Diabetic Retinopathy that caused 30 % reduction in the need for laser therapy with patients with diabetic retinopathy.[8] Fibrates activate ligand activated

transcription factors, peroxisome proliferator-activated receptors (PPARs) especially PPARα that mediate transcription induction of HDL lipoproteins. PPARs are a group of nuclear receptor proteins, that are, responsible for the regulation of genes responsible for important biological processes like glucose homeostasis and Lipid metabolism by binding to specific response elements (PPREs).[9] PPAR initiates transcription of specific genes by binding with retinoid X receptor upon agonist binding and causing a conformational change. PPAR's comprise of three sub-types namely, PPARα, PPARγ, and PPARβ/δ that are expressed in different tissues with differences in the ligand binding domain. Thiazolidinediones are a class of hyperglycemic drugs that act as PPARγ agonists and are used for the treatment of Diabetes Mellitus type 2.[10] TZDs have satisfactory effects on patients in pre-diabetic stage.[11]

Much structurally diverse class of natural and synthetic PPAR agonists has been identified so far. Long chain fatty acids and eicosanoids are endogenous ligands that regulate lipid homeostasis by modulating DNA transcription by binding to specific nucleotide sequences located in the regulatory regions of target genes known as peroxisome proliferator responsive elements (PPREs).[9] Hypolipidemic drugs (fibrates) are reported to perform the same function as these endogenous ligands. Among fibrates, Chlorofibrate and Fenofibrate gained attention in treating Diabetic Retinopathy and other microvascular complications, along with laser treatment. Fenofibrate was identified to regulate the expression of genes responsible for angiogenesis, apoptosis and inflammation. Furthermore, animal studies with Fenofibrate showed to control of neovascularization in the retina of the mouse model.[12] Fibrates, were first known to activate PPARα in 1970's.[13] later, fibrates, fenofibrate, bezafibrate and gemfibrozil have also been shown to inhibit ALR2,[14] which indicate their dual role in the suppression of neovascularisation, by inhibition of ALR2 and activation of PPARγ. ALR2 inhibtors that could also activate PPARγ would be a heuristic approach in the treatment of cancer and diabetes.

MATERIALS AND METHODS

All analysis were performed using Shrödinger LLC-Maestro version 10.2

Preparation of the ligands and PPARγ protein structures for analysis

The crystal structures 2Q5S, 4YT1 and 4EMA corresponding to PPARγ, with resolution 2.05 Å, 2.2 Å, 2.55 Å respectively were retrieved from Protein database (PDB) and prepared using protein preparation wizard of the *Schrödinger* software. The structure-data files (sdf) of ligands reported to inhibit ALR2 and the agonists of PPARγ were retrieved from PubChem database and were structurally minimized using OPLS_2005 force field.

Glide docking of ARIs, Fibrates and full, partial agonist of PPARγ on the structure of PPARγ

Docking studies were performed using Glide module of the schrodinger, on the PPARγ crystal structures with partial and full agonists. Receptor grid was generated based on the binding site information of the protein(s) and the ligand. The crystal structures, 2Q5S, 4YT1 and 4EMA were used as reference structures for the identification of partial and full agonists of PPARγ respectively. The Glide docking interactions of PPARγ with NTzDpa ligand (PDB ID: 2Q5S) and PPARγ with MEKT76 ligand (PDB ID: 4YT1) were exploited to identify partial agonists of PPARγ, and the binding interactions of PPARγ with Rosiglytasone (4EMA) was exploited to identify full agonists of PPARγ. The ligands were docked using Extra-precision (XP) docking strategy. The XP Glide score was used as the fitness function to categorize the ligands according to their binding affinity. The value of the Glide score corresponds to the binding energy in kcal/mol. A low glide score value denotes high affinity of the ligand towards the protein. The ligands were docked to the crystal structure of PPARγ (4EMA) to know the binding interaction of the ligand, relating to the agonistic property of the ligand.

RESULTS

Interaction studies of PPARγ partial and full agonists on PPARγ protein structure

Glide docking interaction studies were carried out to record the binding interactions of the ligand on the PPAR protein, relating to the agonistic activity of the ligand. The known full agonist of PPARγ, Rosiglytasone, interacted with the polar amino acid residues, Ser289 and Hie323 of PPARγ, to establish a conformational change in the LBD to activate PPARγ. Rosiglytasone showed a glide score of -9.255 kcal/mol (Table 1). The partial agonists NTzDpa and MEKT76, established hydrogen bond interactions with Ser342 and MEKT76 had additional hydrogen bond interactions with Ser289, Ser342 and pi-pi interactions with His449 and His266. MEKT76 established a low Glide score value of -11.304 kcal/mol (Table 1), than the other agonists. NTzDpa established a Glide score value of -8.581 kcal/mol (Table 1).

Binding interactions of fibrates on PPARγ

Binding interactions of the full and partial agonists were noted to identify agonists of PPARγ among ALR2 inhibitors. The full agonists, Fibrates, Bezafibrate, Ciprofibrate and Gemfibrozil showed hydrogen bond interactions with the polar residues, Ser289 and hie323, causing a conformational change activating the PPAR protein. The latter had pi-pi interactions with the polar Hie449 residue, corresponding to full agonism. Bezafibrate, the known PPARγ agonist had higher affinity towards the protein with -7.540 kcal/mol Glide score, compared to the PPARα agonists, Ciprofibrate and Gemfibrozil, with -5.677 kcal/mol and -5.366 kcal/mol respectively (Table 2).

Binding interactions of reported ALR2 inhibitors on PPARγ

The binding interactions of ALR2 inhibitors on PPARγ, was similar to that of PPAR agonists. Table 3 lists the Glide docking score and the associated interactions of ALR2 inhibitors on PPARγ. Curcumin and its metabolites, hexahydrocurcumin and tetrahydrocurcumin had higher affinity, corresponding to their low glide scores -8.492 kcal/mol, -10.967 kcal/mol, -9.877 kcal/mol. Curcumin established π-π interaction with Hie449, π-cation interaction with positively charged Arg288 residue and two hydrogen bond interactions with polar Hie323 and hydrophilic Tyr473 residues. Hexahydrocurcumin established π-cation interaction with the positively charged Lys367, hydrogen bond interactions with the main chain hydrophilic aminoacids Ile281, Cys285 and side chain Tyr473 residue. Tetrahydrocurcumin established hydrogen bond interaction with Tyr473. Sesamin interacted with the hydrophobic Tyr327 residue. Catechin had hydrogen bond interactions with the polar Ser342 and Gly284. Rutin had hydrogen bond interactions with the Polar Ser289 residue, Glu291 and positive charged Arg288. (2S)-2'-methoxykurarinone, Epalrestat and Chrysophanol had hydrogen bond interaction with the polar Ser342 residue. 8-lavandulylkaempferol had hydrogen bond interaction with Ser342 and Glu343. Rosmaric acid showed π-cation interaction with Arg288 and hydrogen bond interactions with Tyr473 and Hie323. Cynaroside had hydrogen bond interactions with Ser289, Tyr327 and Glu343. Cirsimarin established hydrogen bond interaction with polar Ser289 and Hie323 residues. Lucidin had hydrogen bond interactions with polar Ser342 and hydrophobic Leu340 residue. Among the glide scores recorded, hexahydrocurcumin and cynaroside had lowest glide scores -10.967 kcal/mol, -10.005 kcal/mol respectively, corresponding to high affinity of the ligands towards the PPARγ protein. The Glide scores of other compounds were between -10.000 kcal/mol to -4.000 kcal/mol. Epalrestat, the known drug used for inhibition of ALR2, had glide score of -4.814 kcal/mol (Table 3).

Table 1: Binding interactions of PPARγ- PPARγ agonists

Compound Name	Protein-Ligand interaction (PPARγ- PPARγ agonists)	Type of agonism	Glide Score (kcal/mol)
Rosiglytasone (4EMA)	SER289, HIE323	Full agonist	-9.255
2Q5S (NTzDpa)	SER342	Partial agonist	-8.581
4YT1(MEKT76)	HIS266, SER289, SER342, HIE449	Partial agonist	-11.304

Table 2: Binding interactions of PPARγ-fibrates

Compound Name	Protein-Ligand interaction (PPARγ- Fibrates)	Type of agonism	Glide Score (kcal/mol)
Bezafibrate	SER289, HIE323	Full agonist	-7.540
Ciprofibrate	SER289, HIE323	Full agonist	-5.677
Gemfibrozil	HIE449	Full agonist	-5.366

Table 3: Binding interactions of PPARγ- ALR2inhibitors

Compound Name	Protein-Ligand interaction (PPARγ-ALR2inhibitors)	Type of agonism	Glide Score (kcal/mol)
Cynaroside	GLU291, SER289, TYR327	Full agonist	-10.005
Cirsimarin	SER289, HIE323	Full agonist	-7.899
Lucidin	SER342, LEU340	Partial agonist	-5.907
Chrysophanol	SER342	Partial agonist	-5.832
Hexahydrocurcumin	ILE281, CYS285, LYS367, TYR473	Full agonist	-10.967
Tetrahydrocurcumin	TYR473	Full agonist	-9.877
Rosemarinic acid	ARG288, HIE323, TYR473	Full agonist	-8.839
Curcumin	ARG288, HIE323, TYR473, HIE449	Full agonist	-8.492
Catechin 5-O-gallate	GLY284, SER342	Partial agonist	-7.084
Rutin	ARG288, SER289, GLU291	Full agonist	-8.813
(2S)-2'-methoxykurarinone	SER242	Partial agonist	-5.070
Epalrestat	SER342	Partial agonist	-4.814
8-lavandulylkaempferol	SER342, GLU343	Partial agonist	-4.652

DISCUSSION

Aldose reductase inhibition related suppression of anti-inflammatory pathways, and neovascularization, the dual action of PPARγ agonists mediating decrease in blood glucose and hypercholesterolemia along with suppression of tumor metastasis has lead to the intervention of ALR2 inhibitors for PPARγ activaltion to prevent diabetic complications and cancers. Thiazolidinediones, are reported to have serious side effects, increasing the requirement of discovering novel ligands that activate PPARγ partially or fully to suppress neovascularization in tumor growth. Discovery of novel ligands that could potentially normalize blood glucose and control hyperglycemia related pathological changes, without side effects would be an effective approach in treating cancer and diabetes. Research on natural ligands for PPARγ activation has obtained positive results from traditionally used medicinal plants.

The ligands have different binding modes and induce a partial activation of the PPARγ receptor and some of which also activate PPARα. Our research on ALR2 natural inhibitors has resulted in identification of the dual action of most of the inhibitors of ALR2 as PPARγ agonists. *Quercetin,* Luteolin, Curcumin, magnolol, honokiol and resveratrol are some of the inhibitors of ALR2[15] that were reported to activate PPARγ.

Full agonists like thiazolidinediones are withdrawn due to their associated side effects. Full agonists Interact with LBD residues S289, H323, Y473, and H449 of the PPARγ gamma. Partial agonists that do not present adverse side effects are encouraged among full agonists. Regulation of PPARγ is dependent on the rate of phosphorylation of Ser273 or its isoform Ser245 at LBD. Binding of the PPARγ agonist Rosiglitazone, to the LBD inhibited CDK5-mediated phosphorylation of Ser273 residue that has altered the expression of regulatory genes involved in the increase in expression of adiponectin, an insulin sensitizing that sensitizes cells to insulin. The antidiabetic property shown by the full agonist, Rosiglitazone might relate to the reduced phosphorylation of Ser273.[16] Activation by full agonists occurs through hydrogen bond interactions between the S289, H323, Y473, and H449 residues of the PPARγ-LBD. Selective PPARγ modulators (SPPARγMs) are partial agonists of PPARγ that bind differently than full agonists.[17] With response to ligand binding, the H12 α-helix in the ligand-dependent activation domain (AF-2) closes the LBD and the change in the confirmation

activates PPARγ, that inturn binds to the co-activator protein and initiates the transcription process of various genes. Full agonists like thiazolidinediones cause a conformational change in the LBD and activates PPARγ. In contrast to the H12-LBD activation with a low rate of H12 involvement and coactivators, partial agonists suppress the transcriptional activity of PPARγ. Research on partial agonists, reveal that they bind to the Ser342 residue of PPARγ with hydrophobic interactions resembling full agonists.[18]

Though synthetic full agonists of PPAR*γ* have been used to treat diabetes, their use has been limited due to the associated severe side effects. This has raised the need for identification of partial agonists of PPAR*γ*, which could hold promise in treating diabetes with less incidence of side effects with potential insulin-sensitizing effects.[19] The natural compounds reported to inhibit ALR2, also had interactions with the critical amino acids responsible for the agonism of PPARγ. Fibrates exibited interactions relating to full agonism of PPARγ. Cynaroside, cirsimarin, hexahydrocurcumin, tetrahydrocurcumin, rosemarinic acid, and curcumin had interactions with the PPARγ protein relating to full agonism. Tetrahydrocurcumin, catechin-5-O-gallate, rutin, (2S)-2'- methoxykurarinone, epalrestat, 8-lavandulylkaempferol had interactions with the PPARγ protein relating to partial agonism. All the ALR2 inhibitors analyzed for PPAR agonism, showed binding interactions related to either full or partial agonist. ALR2 inhibitors that act as partial agonist could provide a dual beneficial effect in treating diabetes and cancers. The ALR2 inhibitors which could act as PPAR partial agonists would be a heuristic approach in treating diabetes and cancers.

CONCLUSION

ALR2 has been implicated in the pathology of diabetic complications, since activation of ALR2 during hyperglycemia, cause series of events producing oxidative stress related cell damage. Fibric acid derivatives, initially reported to inhibit ALR2 and prevent angiogenesis, were proved as PPARγ full agonists. The present work reports that, ALR2 inhibiting ligands, bind to the LBD of PPARγ, similar to partial or full agonists of PPARγ, hence could serve a dual role in controlling neovascularization in diabetes and cancers. The agonist and antagonist role of natural compounds, on two different proteins could be further analysed and the structural similarities between the two proteins, ALR2 and PPARγ could be exploited to identify effective compounds with dual role in the anti-angiogenic treatments.

ABBREVIATION USED

AGE: Advanced glycation end product; **ALR2:** Aldose reductase; **AP-1:** Activator protein 1; **CRC:** Colorectal cancer; **NF-κB:** Nuclear factor kappaB; **PI3K:** Phosphatidyl inositol-3-kinase; **PPARs:** Peroxisome proliferator-activated receptors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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