

The role of peroxisome proliferator-activated receptors in human disease

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ABSTRACT

Increasing attention has been focused on the role of peroxisome proliferator-activated receptors (PPARs) in the past decade. Compelling data have begun to unite work from various arenas, such as epidemiology and vascular biology. Clinical trials with synthetic PPAR agonists have exhibited therapeutic benefits in treating various chronic diseases like atherosclerosis, diabetes mellitus and cardiovascular diseases. The PPARs, a family of nuclear receptors (NRs), are a set of three receptor sub-types encoded by distinct genes. They function as lipid sensors to regulate a broad range of genes in many metabolically active tissues. The discovery of PPAR-specific ligands has led to a significant advancement in our understanding of the structure of these receptor proteins and molecular mechanisms of their ligand dependent activation. Herein, we have tried to delineate the role of PPARs as molecular targets for the development of new drugs to treat human metabolic diseases.

KEY WORDS: PPARs, PPAR ligands, nuclear hormone receptor, metabolic diseases.

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Introduction

Nuclear hormone receptors (NR) are ligand activated transcription factors that regulate gene expression in response to small lipophilic compounds. The well known members of this NR family are testosterone receptor [androgen receptor (AR)], oxysterols receptor [liver X receptor (LXR)], estrogen receptor (ER), xenobiotics receptor [pregnane X receptor (PXR)], retinoic X receptor (RXR), thyroid hormone receptor (TR), bile acids receptor [farsenoid X receptor (FXR)] and vitamin D receptor (VDR).

The peroxisome proliferator-activated receptors (PPARs) comprise an important subfamily of the NR superfamily that plays a central role in the regulation of storage and catabolism of dietary fats.^[1-3] The three subtypes of PPAR (alpha, delta and gamma) bind to fatty acids (FAs) and fatty acid metabolites and regulates the expression of genes involved in the transport, metabolism and buffering of these ligands with cells. Each of the three PPAR subtypes exhibits a unique expression pattern within vertebrate tissues.

PPAR α are more widely expressed in brown adipose tissue, skeletal muscle, heart, liver and kidney than PPAR γ (white and brown adipose tissues, muscle, colon and liver).^[2] Besides, both PPAR α and γ are expressed in the major cellular constituents of the vessel wall (endothelial cells, vascular smooth muscle cells (VSMC) and monocytes/macrophages) as well as human atherosclerotic lesions.^[4] PPAR δ exhibits wide tissue

distribution and is found in all tissues studied to date.^[2]

How do PPARs work at the molecular level?

PPARs possess the canonical domain structure of other NR superfamily members. The functional domains of the PPARs consist of poorly characterised N-terminal region that contain a potential trans-activation function known as activation factor-1 (AF-1), DNA-binding domain (DBD) and ligand binding domain (LBD). Molecular modeling reveals that DBD and LBD, at carboxyl terminus, is a large hydrophobic pocket, which contains a key, ligand dependent trans-activation function called activation factor-2 (AF-2). As described in Figure 1, PPARs bind to cognate DNA elements called PPAR response elements (PPREs) in the 5'-flanking region of target genes. Like many other NRs, they bind DNA as obligate heterodimers by partnering with one of the retinoid X-receptors. Known PPREs are direct repeats of all AGGNC A half site separated by a one base pair spacer.

A short sequence located immediately upstream of the first half site confers polarity on the PPREs, with the PPAR moiety binding 5' to the RXR half of the heterodimer. But many cell types express more than one PPAR isoform. So most likely isoform specific targets are regulated through a combination of subtle cis-sequence differences flanking the core response element, the presence of specific or selective co-activator proteins and regulation of endogenous ligands.^[5]

Like other NRs, PPARs form protein-protein interaction with a variety of nuclear proteins known as co-activators and co-repressors, which mediate contact between the PPAR-RXR heterodimer, chromatin and basal transcriptional machinery, which also promotes activation and repression of gene expression respectively. Co-activator proteins promote the early stage of transcription and fall into three categories.

1. Protein with histone acetylase activity which remodels chromatin structure (e.g. SRC-I, CBP).
2. Members of the DRIP/TRAP complex which interact with basal transcription architecture (e.g. PBP/TRAP220).
3. Proteins with incompletely defined function (e.g. PGCI, RIP 140).^[6]

There are no known receptor specific co-activators or co-repressors, although selectivity for one or the other NR has been illustrated in certain cases and thus may form the basis for tissue specific targets of certain NR ligands.^[7-8] Co-activator proteins either possess or recruit histone acetyl transferase (HAT) activity to the transcription initiation site. Acetylation of histone protein is believed to relieve the tightly packed structure of the chromatin allowing the RNA polymerase II complex to bind and initiate transcription. Co-activators also recruit the chromatin remodeling SWI-SNF complex to target promoters.^[3, 5, 9]

What are the physiological roles played by PPARs?

PPAR α

PPAR α was cloned early in 1990s. It plays an important

role in the oxidation of fatty acids in the liver. Receptor activation stimulates fatty acid oxidation such as in fasting, which is a crucial adaptive response to nutritional challenge. PPAR α is highly expressed in tissues with high rates of fatty acid catabolism. This receptor regulates genes that control fatty acid uptake, causes activation of acyl CoA esters and degradation by way of peroxisomal and mitochondrial β -oxidation pathways. PPAR α activators reduce the quantities of available fatty acids for triglyceride rich very low density lipoprotein (VLDL) synthesis in the liver. So, physiological role of PPAR α receptor is to sense the total flux of dietary fatty acids in key tissues.^[10]

PPAR α ligands

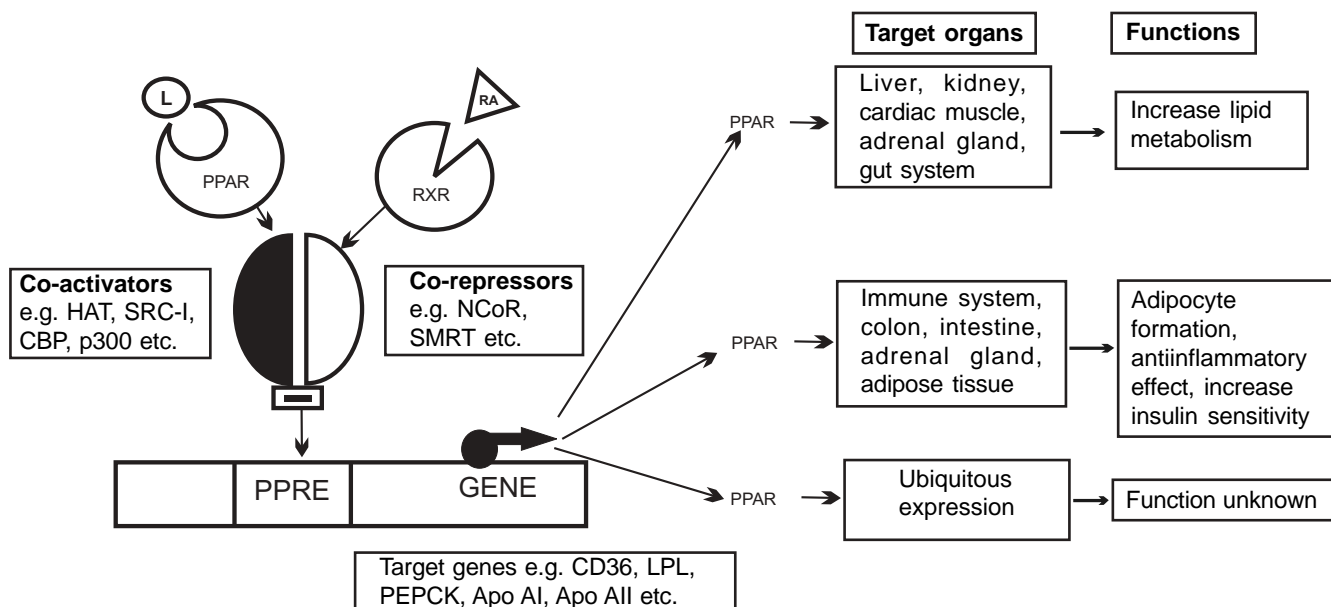
PPAR α binds to a diverse set of ligands, namely, arachidonic acid metabolites (prostaglandins and leukotrienes), plasticisers and synthetic fibrate drugs such as bezafibrate, fenofibrate, clofibrate and gemfibrozil.^[10] More recent thioisobutyric acid compounds (GW 7647, GW 9578) show excellent selectivity for PPAR α receptors.^[11,12] Recently reported LY518674 is a novel selective PPAR α agonist.^[13]

Pharmacological role of PPAR α agonists in human disease

Dyslipidemia

Lipid homeostasis imbalance has been linked to cardiovascular diseases. In addition to obesity, insulin resistance and hypertension are co-morbidities associated with dyslipidemia. In particular, lowering plasma triglycerides (TGs)

Figure 1. The peroxisome proliferators-activated receptors (PPARs) as transcription factor. As members of the steroid hormone nuclear receptor family, PPARs are thought to control gene expression through a heterodimeric complex with the retinoid X nuclear receptor (RXR). Both PPAR and RXR activation are controlled by binding to specific ligands. The ultimate transcriptional response is determined by the association or release of specific co-activators and corepressors. This complex binds to certain PPAR response elements (PPRE) in the promoter regions of target genes controlling their expression, either inducing or repressing the transcriptional response. L=ligand; RA=9-cis-retinoic acid. (NCoR=Nuclear receptor co-repressors, SMRT=Silencing mediator for retinoid and thyroid-hormone receptor, HAT=Histone acetylase transferase, CBP=CREB binding protein, SRC-I=Steroid receptor co-activator, LPL=Lipoprotein lipase, PEPCK=Phosphoenolpyruvate carboxykinase).



and elevating high density lipoprotein cholesterol (HDLc) are of vital importance in reducing diabetic cardiovascular risk. The fibrates are a class of lipid lowering drugs that mediate their clinical effects primarily through activation of PPAR α .^[14,15]

Evidence from studies in rodents and humans implicate 5 major mechanisms underlying the modulation of lipoprotein phenotypes by fibrates.

1. Induction of lipoprotein lipolysis: Increased triglyceride-rich lipoprotein (TRLs) lipolysis could be a reflection of change in intrinsic lipoprotein lipase (LPL) activity or increased accessibility of TRLs for lipolysis by LPL owing to a reduction of TRL apoC-III content.
2. Induction of hepatic fatty acid (FA) uptake and reduction of hepatic triglyceride production: In rodents, fibrates increase FA uptake and conversion to acyl-CoA by the liver owing to the induction of FA transporter protein (FATP) and acyl-CoA synthetase (ACS) activity. Induction of the β -oxidation pathway and ω -oxidation (Cytochrome P450) pathway with a concomitant decrease in FA synthesis by fibrates results in a lower availability of FAs for triglyceride synthesis, a process that is amplified by the inhibition of hormone-sensitive lipase in adipose tissue by fibrates.
3. Increased removal of LDL particles: Fibrate treatment results in the formation of LDLc with a higher affinity for the LDL receptor, which are thus catabolised more rapidly.
4. Reduction in neutral lipid (cholesteryl ester and triglyceride) exchange between VLDL and HDLc may result from decreased plasma levels of TRL.
5. PPAR α activation influences the expression of five key genes encoding for proteins involved in HDLc metabolism. The fibrate class of PPAR α agonist has been shown to increase HDLc synthesis through interaction of gene encoding for apolipoprotein A-I, apolipoprotein A-II and lipoprotein lipase. In addition, PPAR α activators increase 'reverse cholesterol transport' by accelerating the efflux of cholesterol from peripheral cells and increasing its uptake into liver through a pathway involving increased vascular expression of the HDLc receptors, ATP-binding cassette transporter-I (ABC-I) and scavenger receptor class-B type-I (SR-BI).

The overall effect of PPAR α activation on lipid profile is achieved through increased HDLc synthesis, accelerated cholesterol efflux and hepatic uptake, which enhances the HDLc protective effect providing significant clinical benefit.^[2, 3, 9, 16]

Atherosclerosis

PPAR α agonist affects a range of biological processes which contribute to the etiology of coronary artery disease. For instance, expression of VCAM-I (Vascular Cell Adhesion Molecule-I), an adhesive protein which recruits monocytes to endothelial cells at sites of vascular inflammation or atherosclerotic lesions, is downregulated by PPAR α agonist in endothelial cells studies.^[6]

PPAR α is expressed in atherosclerotic plaque and primary culture of smooth muscle cells, macrophages as well as endothelial cells. This inflammatory process can be inhibited

by control of proatherogenic gene transcription induced by NF- κ B (Nuclear Factor- κ B).^[7] Additionally, PPAR α ligands induce apoptosis of macrophages activated with TNF α or γ -Interferon.^[8]

Lipid homeostasis is controlled in part by the nuclear receptor PPAR α and LXR through regulation of genes involved in fatty acid and cholesterol metabolism. Further, recent reports^[17,18] state that pharmacological manipulation of LXR α agonism by PPAR α ligands may provide a novel way to exploit the ability of LXR ligands to prevent/treat dyslipidemia and atherosclerosis.

PPAR α increases the production of the apolipoproteins, apo-AV, apo-CIII, which results in decreased level of TGs in the circulation. Thus, activation of PPAR α reduces the TG content in liver and circulation exactly the reverse of the effects of LXR α activation. Some of the PPAR α agonists also slightly increase HDLc in humans, thus working in the same directions with the LXR α agonist. These results indicate that co-administration of PPAR α agonist may be helpful in eliminating or reducing a significant unwanted effects of LXR α agonists, hypertriglyceridemia. While it may enhance their beneficial effects on the vasculature by increasing HDLc and enlarging HDL particles.^[17,18]

Obesity

Obesity is a risk factor in the development of diabetes and fibrate treatment has been reported to reduce weight gain in rodents. Bezafibrate,^[19] Wy-14643^[20] and other agents^[21,22] induce genes involved in increased energy expenditure of fatty acid catabolism.

Diabetes

Given that these agents have exhibited improved insulin action and glucose utilisation in both high fat fed C57BL6 mice and obese zucker rats, the data suggest that PPAR α ligands can reduce insulin resistance without significant effects on adipose mass accumulation.^[3]

PPAR γ

PPAR γ has been the most extensively studied PPAR subtype to date. Two distinct N-terminal isoforms termed as PPAR γ 1 and PPAR γ 2 have been identified in mice and humans.^[23,24]

PPAR γ is a pivotal transcription factor in the regulation of adipocyte gene expression and differentiation. The regulation of adipocyte differentiation by PPAR γ involves a coordinated signaling cascade with other families of transcription factors. In addition to adipogenic effects, PPAR γ has been shown to be an important regulator of target genes involved in glucose and lipid metabolism. [Table 1] PPAR γ agonists are efficacious antidiabetic agents. PPAR γ agonists may also have therapeutic utility in the treatment of other conditions like atherosclerosis, inflammation and cancer.^[11]

PPAR γ ligands

Ligand studies have shown numerous naturally occurring fatty acids, eicosanoids, prostaglandins and their metabolites to be weak endogenous activators of PPAR γ . PPAR γ exhibits modest preference for essential polyunsaturated fatty acids (PUFAs) including linoleic, linolenic, arachidonic and eicosapentaenoic acids. Thus, PPAR γ may serve as a generalised fatty acid sensor that couples changes in overall PUFAs' concentration with the target genes associated with lipid and glucose homeostasis.^[3]

Table 1**PPAR-regulated genes**

<i>Genes</i>	<i>Functions</i>
Lipoprotein Lipase (LPL), CD36 (Fatty acid transporter; FAT)	Fatty acid incorporation
Acyl-CoA synthetase, Malic enzyme (ME), Phosphoenolpyruvate carboxykinase (PEPCK)	Fatty acid synthesis
Intestinal liver fatty acid binding protein (FABP), aP2 adipose FABP, Microsomal triglyceride transfer protein (MTP), ApoAI, Apo AII, Apo CIII	Lipid transporter
Acyl-CoA oxidase, Ketoacyl-CoA thiolase, Enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase	Beta-oxidation
Mitochondrial 3-hydroxyacyl-CoA synthetase	Ketone-body production
Cytochrome P450, CYP4A1, CYP4A6, CYP4A11	Omega oxidation
HMG-CoA synthase, ATP-binding cassette transporter A-1 (ABCA-1), Scavenger receptor class B type-1 (SR-B1), SR-A, Cholesterol 7 α -hydroxylase (CYP7A1)	Cholesterol, bile acid metabolism
Plasminogen activator inhibitor type-1(PAI-1)	Plasminogen control
Leptin (ob-gene)	Satiety
Uncoupling protein (UCP) 1,2,3	Thermogenesis
Glut 2, Glut 4, Glut 1, c-Cbl associating Protein (CAP)	Glucose transporter
Resistin, Tumor necrosis factor (TNF)- α , Interleukin (IL)-6, Adiponectin	Insulin sensitivity
11 β -Hydroxy steroid dehydrogenase-1 (11 β -HSD-1)	Glucocorticoid secretion

Synthetic agonists

The thiazolidinedione (TZD) class of antidiabetic agents commonly referred to as glitazones' represent the first compounds identified as high affinity PPAR γ agonists such as rosiglitazone, pioglitazone etc. A series of tyrosine based PPAR γ agonists G1262570 (farglitazar), GW 1929 and GW 7845,^[25-27] the isoxazolidinedione JTT- 501,^[28] the TZD analogue KRP-297^[29] have been some of the recently developed compounds showing promising anti-diabetic activity in animal studies.

Selective PPAR γ modulators

Clinical benefits of PPAR γ agonists in treating type-2 diabetes has been clearly demonstrated, but the problem associated with current generation of glitazone drugs is that they are associated with undesirable side effects such as weight gain and edema.

Thus, it was of significant interest to design PPAR γ modulator, which retains efficacious insulin sensitising

Table 2**Endogenous ligands of PPARs^[3,5]****PPAR α agonist**

Arachidonic acid metabolites (PGs and LTs)

PPAR γ agonist

Naturally occurring fatty acids

Eicosanoids and its metabolites

Prostaglandins (e.g. 15-deoxy- +12,14 – prostaglandin J₂) and its metabolites

Essential Polyunsaturated fatty acids (PUFAs) (e.g. linoleic acid, linolenic acid, arachidonic acid and eicosapentaenoic acid)

Oxidized metabolites of PUFAs (e.g. 9 or 13 – hydroxyoctadienoid acid 9-HODE or 13-HODE)

PPAR δ agonist

Saturated and unsaturated fatty acids

properties while minimising potential adverse effects. GW 0072 (non TZD thiazolidine acetamide) antagonises the adipocyte differentiation induced by rosiglitazone but promotes adipocyte differentiation in the presence of insulin and, hence, functions as insulin sensitiser.^[30,31] It can inhibit the adipogenic effects of rosiglitazone but not insulin, without inducing much weight gain. Glitazones MCC-555 (netoglitazone) and NC-2100 represent a second class of PPAR γ modulators.^[32,33]

Antagonist

Recently, many irreversible PPAR γ antagonist ligands have been identified namely GW9662, T0070907, PD068235, LG100641, BADGE and so on.^[34] These PPAR γ antagonists block adipogenesis induced by either rosiglitazone or insulin by inhibiting transcriptional activity and co-factor association induced by rosiglitazone. GW9662 was recently identified as irreversible PPAR γ antagonist ligand. It acts as PPAR γ antagonist at concentration of 1-10 μ M in cell-based assay. It binds covalently to the Cys 286 gene, located on helix three of the PPAR γ ligand-binding domain. It displays greater affinity for PPAR γ than for PPAR α or PPAR δ although this cysteine residue is conserved in all three PPAR subtypes. It antagonises PPAR α activation in multiple cell lines including adipocytes, macrophages and hepatic stellate cells.^[34-37] The ligand LG100641 has been described as a specific antagonist that inhibits rosiglitazone induced adipocyte differentiation but stimulates insulin mediated glucose uptake in adipocytes.^[38] The plasticizer BADGE blocks both rosiglitazone and insulin induced adipogenesis but requires concentrations that approach its limit of solubility.^[31] The PPAR γ ligand CDDO-Me is a synthetic triterpenoid that has been shown to inhibit adipocyte differentiation at concentration below 1 μ M.^[31]

Pharmacological role of PPAR γ agonists in human disease*Diabetes*

The treatment of type-2 diabetes is the most widely studied therapeutic utility for a PPAR γ agonist. PPAR γ agonists reduce plasma glucose, lipid and insulin levels in type-2 diabetes.^[39,40]

TZDs are the new class of drugs useful in the treatment of type-2 diabetes. Recent advances include the discovery of novel

Table 3**Exogenous ligands of PPARs**

<i>Compound</i>	<i>Status</i>	<i>References</i>
PPARα agonists		
Clofibrate	Marketed	10
Fenofibrate	Marketed	10
Bezafibrate	Marketed	10
Gemfibrozil	Marketed	10
WY 14643 (Wyeth Pharmaceuticals)	Preclinical stage	3
GW 7647 (Glaxo Smith Kline)	Preclinical stage	11
GW 9578 (Glaxo Smith Kline)	Preclinical stage	11
LY 518674 (Eli Lilly & Co.)	Preclinical stage	13
PPARγ agonist		
Rosiglitazone (Glaxo Smith Kline)	Marketed	3
Pioglitazone (Kyorin Pharmaceuticals)	Marketed	3
KRP-297 (Kyorin Pharmaceuticals, Merck)	Phase I	29
GW 1929 (Glaxo Smith Kline)	Preclinical stage	3, 25-27
GW 7845 (Glaxo Smith Kline)	Preclinical stage	25-27
L-165041 (Eli Lilly & Co.)	Preclinical stage	3
Ciglitazone	Withdrawn from Market.	3
Troglitazone (Glaxo Smith Kline)	Withdrawn from Market.	3
JTT-501 (Japan Tobacco Inc.)	Terminated	28
PPARγ antagonist		
MCC 555 (Mitsubishi, J & J)	Phase II	32-33
GW 9662 (Glaxo Smith Kline)	Preclinical stage	3, 34
T 0070907 (Tocris, Sankyo, Tularis)	Preclinical stage	3
LG 100641 (Ligand Corporation)	Preclinical stage	38
NC 2100 (Nippon Chemiphar)	Preclinical stage	32-33
GW 0072 (Glaxo Smith Kline)	Preclinical stage	30-31
BADGE	Investigational tool	3
PD 068235 (Pfizer Global R & D)	Investigational tool	3
CDDO-Me (Glaxo Wellcome R & D)	Investigational tool	3
PPARδ ligands		
GW 501516 (Glaxo Smith Kline)	Preclinical stage	69
GW 0742 (Glaxo Smith Kline)	Preclinical stage	76
L-165041 (Merck)	Preclinical stage	77
PPARα and γ agonist (Dual PPAR agonists)		
Muraglitazar (Bristol Mayer, Merck)	Phase III	87,89
Tesaglitazar (Galida) (AstraZeneca)	Phase III	3,89,91
LY 929 (Eli Lilly & Co., Ligand Corporation)	Phase I	89
LSN 862 (Eli Lilly & Co.)	Preclinical Stage	88
Ragaglitazar (Dr. Reddy's Lab.)	Terminated	3
PPARα, γ and δ (Pan) Agonist		
GW 677954 (Glaxo Smith Kline)	Phase II	89
PLX 204 (Plexxikon Corp)	Phase I	89
DRL-11605 (Perlecan Pharma)	Phase I	93

genes that are regulated by PPAR γ , which helps explain how activation of this adipocyte predominant transcription factor regulates glucose and lipid homeostasis. Increased levels of circulating free fatty acids (FFAs) and lipid accumulation in nonadipose tissue have been implicated in the development of insulin resistance. This situation is improved by PPAR γ ligands which promote fatty acid storage in fat depots and regulate the expression of adipocyte secreted hormones that impact on glucose homeostasis.

Adiposa is a major target tissue of insulin sensitising PPAR γ ligands. However, improved glucose homeostasis related to administration of PPAR γ ligands such as TZDs involves insulin sensitisation in muscle and liver, which raised the paradoxical question, "How does a receptor, expressed predominantly in adipose tissues, improve glucose metabolism in muscle?"^[41] TZDs suppress insulin resistance in adipose tissue in addition to skeletal muscle and liver, which contain low concentration of PPAR γ . Adipose tissues function as an endocrine organ.

PPAR γ ligand increases the expression of cell surface receptors such as fatty acid protein transporter (FATP), CD36 and others, which results in trapping and uptake of FFA in adipocytes. In addition to increased FFA flux into adipocytes, FFA efflux is reduced by the expression of genes which promote the storage of FFAs in the form of TGs, by increasing production of glycerol-3-phosphate. The PPAR γ ligand also expresses other genes which are necessary for triglyceride synthesis such as phosphoenolpyruvate carboxykinase (PEPCK), glycerol kinase (GyK) and so on. [Table 1]

Consequently, lipid levels in adipose tissue rise whereas circulating FFAs diminish.^[42] So, by repartitioning lipid away from liver and muscle, PPAR γ agonists ameliorate hyperglycemia, by reversing lipotoxicity induced insulin resistance. Data from patients with type-2 diabetes mellitus and preclinical studies also demonstrate that PPAR γ agonists function as 'adipose remodeling factors' that redistribute lipids from insulin-resistant, lipolytic visceral-fat depots into subcutaneous fat that contains small, newly differentiated, insulin-responsive adipocytes.

Adipocyte-derived leptin is a circulating regulator of appetite and energy expenditure. Adiponectin and resistin are additional adipocyte-specific secreted proteins which appear to have a role in insulin sensitivity as do other polypeptides which are secreted non-exclusively by adipocytes, including TNF α , plasminogen activator inhibitor 1 (PAI-1) and IL-6. TZDs repress adipocyte gene expression of resistin, TNF α and IL-6, all of which have been implicated in insulin resistance. Furthermore, TZDs decrease adipocyte secretion of PAI-1, a prothrombotic which is increased in obesity. Moreover, PPAR γ ligands induce gene expression of adiponectin, insulin sensitising adipocyte hormone. Remarkably, gene regulation as a result of TZDs treatment alters many, if not all, of these adipose derived endocrine factors. Overall, therefore, PPAR γ ligands alter the expression of several adipocyte hormones in a manner that is likely to mitigate insulin resistance.^[41]

PPAR γ ligands regulate the expression of several other genes that enhance glucose metabolism in the adipocyte, including those which encode the insulin responsive glucose transporter GLUT4, GLUT2 and c-Cbl associating protein (CAP) (crucial for GLUT4 translocation to the surface). By this way, they increase glucose uptake into tissue and decrease overall glucose. Overexpression of 11 β -HSD1 (11 β -hydroxy steroid dehydrogenase1) in adipocytes cause insulin resistance, suggesting that reduction of adipocyte 11 β -HSD1 might promote insulin sensitivity, either by reducing glucocorticoid induced gene expression in the adipocyte or by reducing adipocyte secretion of glucocorticoids. Any or all of these effects might contribute to the smaller adipocyte size that is associated with PPAR γ activation. It has been reported that smaller adipocytes typically have greater insulin sensitivity, take up more glucose and have lower rates of lipolysis compared to large adipocytes.^[3,43,44]

Atherosclerosis

The progression of atherosclerosis involves the accumulation of foam cells underneath the arterial wall endothelium. Foam cells are cholesterol laden macrophages which result from the internalisation of oxidised LDL (oxLDL) particles by lipid transporters such as CD36, SR-A and

others.^[45,46] The abundant expression of PPAR γ might seem that PPAR γ is proatherogenic by promoting foam cell formation. However, using a standard model of atherosclerosis Li *et al*^[46] had demonstrated that the treatment of LDLc receptor deficient mice with rosiglitazone or GW 7845 was shown to prevent the formation of atherosclerotic lesions despite increasing CD36 expression. Several studies have found that the PPAR γ agonist (troglitazone) decreased atherosclerosis when given to either LDLc receptor or apolipoprotein E receptor deficient mice.^[47] In addition, previous clinical data indicates that PPAR γ agonist (troglitazone) actually protects type-2 diabetic patients from atherosclerosis.^[48]

A recent study has provided insight into the antiatherogenic effects of PPAR γ . ABCA-I is a member of the ATP binding cassette family of energy dependent transporter proteins which regulates cholesterol efflux from macrophages. ABCA-I gene expression is regulated by the nuclear oxysterol receptor LXR. PPAR γ activation induces the expression of LXR, which promotes ABCA-I expression and ultimately cholesterol efflux.^[49,50] Thus, PPAR γ may be antiatherogenic *in vivo* by enhancing cholesterol efflux from macrophages and endothelial cells.

In vitro, animal model and clinical studies indicate that TZDs:

- Correct endothelial dysfunction.
- Suppress chronic inflammatory processes.
- Reduce fatty acid formation.
- Delay plaque evolution and vessel wall thickening.
- Enhance plaque stabilisation and regression.

Thus, TZDs show potential as potent antiinflammatory, antithrombotic agents which could improve glucose tolerance and the long-term cardiovascular risk related to atherosclerosis in patients with type-2 diabetes.^[51]

Inflammation

Several investigators have established PPAR γ expression in monocytes/macrophages and human atherosclerotic lesions.^[50,52,53] Jiang *et al*^[54] found that PPAR γ agonists decreased production of tumor necrosis factor- α (TNF- α), interleukin-1 β and interleukin-6 by phorbol 13-myristate 12 acetate, but not lipopolysaccharide stimulated monocyte like cell lines. Ricote *et al*^[55] found that PPAR γ activators decreased the promoter activity for genes such as inducible nitric oxide synthase and matrix metalloproteinase-9 (gelatinase- β). Rosiglitazone, a PPAR γ agonist, was found to be a potent antiinflammatory agent in animal models of acute inflammation.^[56] *In vitro* reports find PPAR γ inhibition of monocyte chemoattractant protein-I directed chemotaxis. PPAR γ agonists also inhibit chemokines (interleukin-8) in epithelial cells, leading to the suggestion of their use in inflammatory bowel diseases.^[3]

Cancer

PPAR γ is highly expressed in several human cancer cell lines, including liposarcoma,^[57] breasts,^[58] colon,^[59] lungs,^[60,61] prostate,^[62] bladder^[63] and gastric.^[64] The PPAR γ agonists such as TZDs and 15d-prostaglandin J₂ (15d-PGJ₂) have demonstrated not only apoptosis and growth inhibition of numerous cancer cell lines *in vitro*, but have also shown tumour growth suppression *in vivo* rodent carcinoma models.^[65,66] Promising phase 2 clinical trials, with troglitazone, suggest

that the use of these agents offer an improved therapeutic treatment opportunity for inoperable lipocarcinomas.^[67]

PPAR δ

Human PPAR δ was cloned in the early 1990s.^[68] Understanding the biological function of PPAR δ , however, has been impeded due to its ubiquitous expression, absence of potent and selective ligands and the lack of connection of clinical disorders. However, growing evidence suggests that PPAR δ plays a role in lipid metabolism, cholesterol efflux,^[69] adipogenesis,^[70] colon cancer,^[71] bone metabolism,^[72] embryo implantation^[73] and development of brain and skin.^[74,75]

PPAR δ ligands

The 4-thiosubstituted phenoxyacetic acids GW501516,^[69] GW0742^[76] are potent and selective PPAR δ agonists. Merck has also reported phenoxyacetate L-165041^[77] and a series of 4-[(aryloxy) propyl thio] phenyl acetate based agents as agonists of PPAR δ .^[78]

Pharmacological role of PPAR δ agonists in human diseases

Lipid metabolism and cholesterol efflux: PPAR δ selective ligands have been reported to increase HDLc in diabetic db/db mice without effects on blood glucose and triglyceride concentration.^[79]

A potent ligand, GW501516, has been shown to induce substantial dose dependent increase in HDLc while lowering LDLc, TGs and insulin levels in insulin resistant middle aged obese rhesus monkeys.^[69]

Adipogenesis

PPAR δ may also play a role in adipocyte differentiation. A recent evaluation of the role of PPAR δ in adipogenesis has revealed that over expression of PPAR δ in NIH-3T3 fibroblasts, in the presence of cAMP elevating agents, induces PPAR γ 2 expression and terminal adipocyte differentiation. However, the PPAR δ selective agonist L-165401 only produces modest terminal differentiation in synergy with cAMP elevating agents in 3T3-L1 preadipocytes.^[70] Studies have shown that PPAR δ deficient mice are smaller and leaner with increased expression levels of fatty acid translocase protein (CD36/FAT) in adipose tissues.^[74] The same studies have shown that independent PPAR δ activation is insufficient to drive terminal adipocyte differentiation, but does promote PPAR γ gene expression, which upon specific ligand activation promotes adipogenesis.

Colorectal cancer

PPAR δ has been implicated as a direct target and potential oncogenic effector of β -catenin in colorectal carcinogenesis in mice.^[80] NSAIDs such as sulindac can antagonise PPAR δ activated gene transcription and reportedly suppress colorectal tumorigenesis.^[81] This suggests that NSAIDs may inhibit tumour formation by blocking PPAR δ activation. Additional studies, employing genetically manipulated human colon cancer cells, have demonstrated that PPAR δ null cells exhibit decreased tumorigenesis in nude mice compared to PPAR δ ^{+/-} and wild type controls.^[81] This evidence suggests that PPAR δ expression may promote tumour growth and thus may be a potential target for the treatment of colorectal cancer.

Bone metabolism

Mature osteoclasts modulate bone resorption activity. High expression of PPAR δ has been identified in mouse and rabbit osteoclasts. The PPAR δ agonist carbaprostacyclin induces bone-resorbing activity. Osteoclastic genes, including

cathepsin K and carbonic anhydrase type II, are also significantly up regulated. These results suggest that PPAR δ may play a key role in osteoclastic bone resorption and PPAR δ antagonists may have potential utility in treating osteoporosis.^[82]

Role of PPARs in CNS

In contrast to normal astrocytes, the cell lines of malignant astrocytoma express higher levels of PPAR γ . This finding tempted scientists to explore the role of PPARs in glial tumours of the brain. Incubation of malignant astrocytoma cell lines with PPAR γ agonists, ciglitazone and 15d-PGJ₂, reduced cell viability and increased apoptotic rate. This may suggest the role of PPAR in regulation of the apoptotic process of astroglial cells.^[83] Other studies also demonstrated that ligands of the PPAR γ induce apoptosis in activated T-lymphocytes and exert antiinflammatory effects in glial cells. Preclinical studies have shown that the TZD pioglitazone delays the onset and reduces the severity of clinical symptoms in experimental autoimmune encephalomyelitis in animal model of multiple sclerosis. Supporting the above observations, Pershadsingh *et al*^[84] reported that daily treatment with pioglitazone (45 mg) for 3 years induced apparent clinical improvement, without adverse events in a patient with secondary progressive multiple sclerosis.

PPAR γ agonist and pan agonist treatment accelerated the differentiation of oligodendrocyte in mixed glial cultures. It implies that PPAR γ plays a significant role in the maturation of oligodendrocyte and regulates the size of oligodendrocyte sheets. The above findings suggest that PPAR γ increases the survival of cells and/or prevents cell death in the enriched culture. However, the role of PPAR γ as a factor in the transcriptional regulation of oligodendrocyte differentiation still needs to be investigated.^[85]

Dual PPAR (α and γ) and Pan PPAR Co-agonism

Diabetic patients are prone to increased risk of coronary heart disease that stems from cardiovascular risk factors such as dyslipidemia, coagulopathy, hypertension and obesity. The hallmark problems of hyperglycemia and insulin resistance are also contributing factors. In general, PPAR γ agonists, the antihyperglycemic agents, provide minimal protection against the eventual cardiovascular risks which develop with type-2 diabetes.^[86] These dual-acting PPAR agonists are a novel group of compounds which also activate nuclear transcription factors. The examples are muraglitazar,^[87] farglitazar, ragaglitazar, reglitazar, tesaglitazar,^[3] LSN 862,^[88] LY 929^[89] and so on. By activating both PPAR α and PPAR γ receptors, they simultaneously reduce atherogenic triglycerides, raise cardioprotective HDL levels and improve insulin resistance. Thus, they address many of the core features seen in people with metabolic syndrome and may help to reverse the underlying disease process and its adverse clinical sequelae, which includes cardiovascular disorders (CVD) and diabetes. Furthermore, the stimulation of lipid catabolism by PPAR α activation may offset PPAR γ induced adipogenesis and thereby diminish the undesired side effect of adiposity that arises from selective PPAR γ stimulation.^[3] Recently, the first dual-PPAR agonist, muraglitazar, has been approved by the US Food and Drug Administration (FDA) advisory committee. However, Nissen *et al*^[87] have reviewed the clinical trial documents and

reported a high incidence of cardiovascular complications and death following muraglitazar administration when compared with placebo or pioglitazone.

The modification at the N-alpha position of the tyrosine-based PPAR γ agonist farglitazar led to the discovery of GW9544, a dual PPAR α/γ agonist with sub-nanomolar potency at PPAR γ .^[12] Studies of novel, PPAR α/γ acting α -ethoxyphenylacetic acids have furnished the Phase III antidiabetic agent ragaglitazar (NN-622). Ragaglitazar exhibits better plasma glucose and triglyceride reduction than rosiglitazone in insulin resistant db/db mice.^[90] Tesaglitazar (Galida), another α -ethoxyphenylacetic acid based PPAR α/γ ligand, appears to improve macrophage export of cholesterol to HDL. The compound increases the reduced HDL-mediated cholesterol efflux to control levels in human, fat exposed monocyte THP-1 cells differentiated into macrophages. Tesaglitazar is poised to enter Phase III clinical trials as it has potential for the treatment of glucose and lipid abnormalities associated with type-2 diabetes and the metabolic syndrome.^[3,91] BMS-298585 is an oxybenzylglycine with potent and selective, balanced PPAR α and γ agonist effects.^[3]

However, safety will be a critical issue with this new class of drugs as several promising candidates have already failed because of adverse toxicity profiles. Adverse effects, seen with some dual-acting PPAR agonists in advanced-stage development, have included oedema, raised levels of hepatic enzymes and tumours in rodents. The casualties include ragaglitazar, reglitazar and, most recently, MK-767, the development of which has been discontinued.^[91]

The old and well-known lipid-lowering fibric acid derivative bezafibrate is the first clinically tested pan (α , β/δ and γ) PPAR activator. It is a sole pan PPAR activator with more than a quarter of a century of therapeutic experience with a good safety profile. Therefore, bezafibrate could be considered as a prototype of a clinically tested pan-PPAR ligand. In patients, with relevant metabolic abnormalities, it is expected to improve both insulin sensitivity and the blood lipid profile and probably reduces the risk of long-term cardiovascular complications. In addition, we can expect prevention of overweight development due to its PPAR β/δ properties.^[92] Recently, GSK and Plexxikon Inc. discovered the novel pan agonists, GW 67795^[89] and PLX 204^[89] which, besides having α and γ dual agonistic activity, also possess δ activity. This α , γ and δ agonistic activity demonstrates highly significant improvement in lipid metabolism. The pan PPAR activator DRL 11605^[93] was discovered under Dr. Reddy's drug discovery programme, wherein the molecule completed its pre-clinical trials and later on was transferred to Perlecan Pharma, Canada, which has commenced Phase I clinical trials.

Toxicity related to PPARs

The role of PPAR ligands has been well established in some very important therapeutic areas such as diabetes, obesity, cardiovascular diseases, inflammation and so on. But, more recently, it is becoming clear that they are also involved in carcinogenesis. Recently, troglitazone showed liver toxicity and hence was pulled out from the market.^[94] Dr. Reddy's ragaglitazar, when studied for long-term rodent toxicity, revealed that it was associated with bladder cancer. Merck, which has a compound from Japan's Kyorin, pulled it out when

another rare cancer showed up in long-term rodent studies. Guidelines recently issued by FDA require that clinical trials of PPAR ligands of greater than 6 months duration be preceded by 2-year carcinogenicity studies in rodents. Although this regulatory change introduces significant delay and complexity into the development of new PPAR agents, work in this area appears to continue unabated.^[42] Whether PPAR ligands produce toxicity via a receptor-dependent and/or off-target mediated mechanism is not yet known.

It is believed that increase in oxidative stress and proliferation (by decreasing the rate of apoptosis) due to activation of peroxisome proliferators (PPs) by ligand binding to PPAR alpha leads to hepatocellular adenoma and carcinoma in rodents. However, there is no evidence that humans are at any increased risk of liver cancer after chronic activation of PPs by PPAR alpha. Epidemiological studies have not revealed any risk of liver cancer development in patients chronically exposed to the widely used hypolipidemic agents gemfibrozil and clofibrate,^[83] i.e. humans appear to be resistant to the induction of peroxisome proliferation and the development of liver cancer by fibrate drugs. The molecular basis of this species difference is not known. To examine the mechanism determining species differences in PPAR response between mice and humans, a PPAR-alpha humanised mouse line was generated in which the human PPAR-alpha was expressed in liver under control of tetracycline responsive regulatory system. The PPAR-alpha humanised and wild-type mice responded to treatment with the potent PPAR-ligand WY-14643 as revealed by induction of genes encoding peroxisomal and mitochondrial fatty acid metabolizing enzymes and resultant decrease of serum TG. However, surprisingly, only the wild type mice and not the PPAR-humanised mice exhibited hepatocellular proliferation, as revealed by elevation of cell cycle control genes and hepatomegaly.

These studies established that following ligand activation, the PPAR-mediated pathways controlling lipid metabolism are independent from those controlling the cell proliferation pathways. These findings also suggest that structural differences between human and mouse PPAR are responsible for the differential susceptibility to the development of hepatocarcinomas observed after treatment with fibrates. Studies reveal that the human PPAR, which has been isolated, has four amino acid differences or lacks exon 6 (alternate RNA splicing) from the wild-type sequence. Whether and how these mutants act as dominant-negative repressors of peroxisomal and/or lipid homeostasis gene expression are unknown. Hence, the extensive information which has accumulated on the mechanism of PP action in rodents, and the response of humans to these compounds, has yet to provide a definitive explanation for species differences. It is unlikely that a single receptor alone will elicit such a complex pleiotropic response but likely, rather, that other mediators are required for the changes in growth, lipid perturbation and peroxisome proliferation. Genes associated with cell survival and proliferation, such as TNF alpha, is under investigation as potential candidates. This cytokine has received a great deal of interest of late as it cannot suppress apoptosis and induce DNA synthesis in a manner similar to PPs.^[95]

Conclusion

The PPAR family of nuclear receptors functions to regulate a broad range of genes in many metabolically active tissues. The PPAR γ agonists have demonstrated insulin sensitising and glucose regulating activity, whereas PPAR α agonists have lipid/cholesterol modulating properties. In addition, PPAR δ plays a significant role in various clinical disorders but its exact physiological importance needs to be elucidated. These receptors participate in the systemic regulation of lipid metabolism acting as sensors for fatty acids, eicosanoids, prostaglandins and related metabolites. PPARs are found to be critical regulators of inflammatory responses, not only through metabolic effects, but also through their direct actions on vascular and inflammatory cells.

These observations point to the fact that PPARs have a therapeutic role in limiting atherosclerosis or its complications. Although, PPARs have emerged as therapeutic targets in treating diabetes and cardiovascular diseases, additional insight into the role of these intrigued receptors in other diseases remains an area of active research. The amount and breadth of research efforts devoted to these proteins ensures that more discoveries are certain to emerge.

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- ❖ A candidate can only apply for one award in a given year

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