

Peroxisome Proliferator-Activated Receptors (PPAR_S): A Target with a Broad Therapeutic Potential for Human Diseases: An Overview

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Summary

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.

Keywords- Nuclear hormone receptor, metabolic diseases, PPARs, PPAR ligands,

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Introduction-

In the field of molecular biology, the peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis of higher organisms. The peroxisome proliferator-activated receptors (PPARs) are ligands-activated intra-cellular transcription factors that have been implicated in important biological processes such as inflammation, tissue remodeling and atherosclerosis. Three types of PPARs have been identified: alpha, gamma, and delta (beta):

- α (alpha) - Expressed in liver, kidney, heart, muscle, adipose tissue, and others.
- β/δ (beta/delta) - Expressed in many tissues but markedly in brain, adipose tissue, and skin.
- γ (gamma) - although transcribed by the same gene, this PPAR through alternative splicing is expressed in three forms:
 - γ_1 - expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas, and spleen.
 - γ_2 - expressed mainly in adipose tissue (30 amino acids longer).
 - γ_3 - expressed in macrophages, large intestine, white adipose tissue [1].

PPAR family consists of 3 subtypes of proteins encoded by separate genes: PPAR α (NR1C1), PPAR β/δ (NR1C3), and PPAR γ (also known as γ or NR1C2). They act as heterodimers with the retinoid X receptor and regulate gene transcription by binding to specific response elements in the promoter of the target genes. The classical biological activity of PPAR α is the regulation of the rate of fatty acid uptake and their esterification into triglyceride or oxidation whereas PPAR γ is classically involved in adipocyte differentiation, regulation of fat storage, and maintenance of glucose homeostasis. The physiological functions of PPAR δ are instead still unclear, although it is known that this receptor contributes to an inflammatory switch through its association and disassociation with transcriptional repressors. The clinical importance of PPARs originates with fibrates

and thiazolidinediones (TZDs), which respectively act on PPAR α and PPAR γ and are used to ameliorate hyperlipidemia and hyperglycemia in subjects with type 2 diabetes mellitus (T2DM). Fibrates, such as gemfibrozil, clofibrate, fenofibrate, and bezofibrate, are drugs that effectively reduce triglycerides (TG) and free fatty acids (FFA) and increase high-density lipoproteins-cholesterol. Fibrates also improve glucose tolerance in T2DM patients, although this activity might be attributable to the fact that some of these compounds also have potential PPAR γ activity. TZDs, such as rosiglitazone, pioglitazone, troglitazone, and ciglitazone, are insulin-sensitizing drugs and have constituted a major advance in the recent therapeutic management of T2DM. In addition to improving insulin sensitivity, TZDs have also effects on TG, FFA, and ketone body level in several animal models of T2DM. The role of PPAR δ remained unclear for almost a decade after its cloning in 1992. Its near-ubiquitous tissue expression raised early speculation that it may serve a “general housekeeping role.” More recently, receptor Knockouts revealed multiple developmental and homeostatic abnormalities in PPA δ null mice, including placental defects causing embryonic lethality, decreased adipose mass, myelination defects, altered skin inflammatory response, and impaired wound healing [2-4]. In recent years, there has been increasing appreciation of the fact that, in addition to the classical biological activities mentioned above; PPARs have several other pleiotropic functions. For instance, they provide a fundamental contribution to the regulation of certain physiological activities of the prostacyclin (PGI₂) system in cardiovascular tissues. Indeed, PGI₂, which is the most abundant product of arachidonic acid in vascular tissues, acts through a dual signaling pathway, that includes both G protein– coupled cell surface receptors named IP and PPARs. In this respect, it has been recently demonstrated that the angiogenic abilities of stable analogues of PGI₂ depend on their capacity to act on PPARs. Additional evidence also suggests that selective activation of PPAR α and PPAR γ promotes a robust angiogenic process in vitro and in vivo, through a mechanism that depends on the stimulation of the prototypical angiogenic agent vascular endothelial growth factor (VEGF). Other data from our group also demonstrate that the ability of PGI₂ analogues to induce angiogenesis depends on the presence and proper function of the PPAR α gene [5, 6].

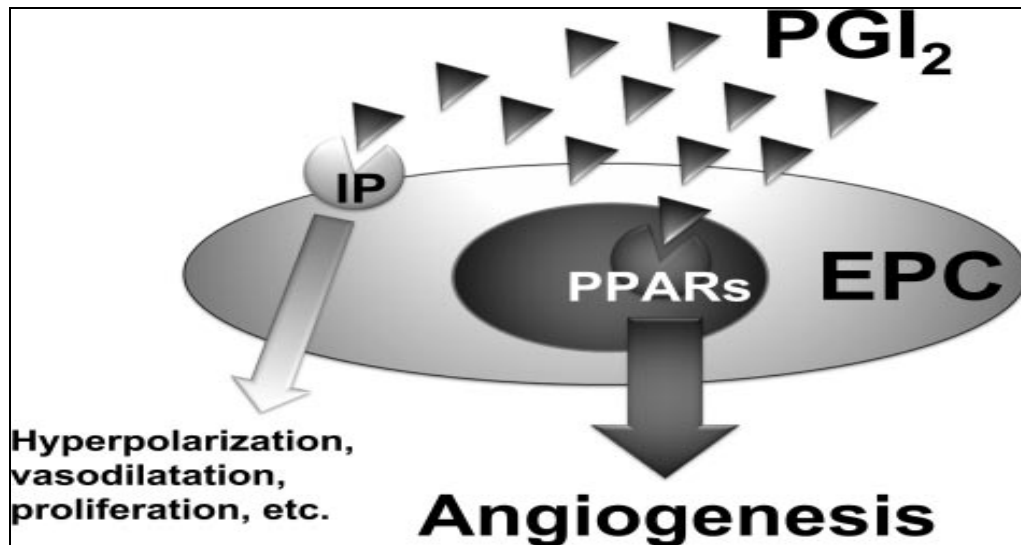


Figure-1 A schematic representation of PGI₂ activity on EPCs. The IP signaling mediates several effects, such as hyper polarization, vasodilatation, and proliferation, but the PPAR signaling is crucial for angiogenic process.

The intersection between the PGI₂ system, PPARs, and angiogenesis is intriguing and deserves further investigation. PPAR pleiotropy is another interesting field of research that might potentially improve our future understanding of the clinical effects of drugs that stimulate PPARs and are currently used in the clinic, with potentially important unexpected implications for the management of subjects with diabetes, hyperlipidemia, and ischemic cardiovascular diseases [7].

PPAR_s- Drugs Modulators-

PPAR α and PPAR γ are the molecular targets of a number of marketed drugs. The three main classes of PPAR drugs are:

PPAR-alpha modulators- PPAR-alpha is the main target of fibrate drugs, a class of amphipathic carboxylic acids (clofibrate, gemfibrozil, ciprofibrate, bezafibrate, and fenofibrate). They were originally indicated for cholesterol disorders (generally as an adjunctive to statins) and more recently for disorders that feature high triglycerides.

PPAR-delta modulators- PPAR-delta is the main target of a research chemical named GW501516. It has been shown that agonism of PPAR-delta changes the body's fuel preference from glucose to lipids.

PPAR-gamma modulators- PPAR-gamma is the main target of the drug class of thiazolidinediones (TZDs), used in diabetes mellitus and other diseases that feature insulin resistance. It is also mildly activated by certain NSAIDs (such as ibuprofen) and indoles. Known inhibitors include the experimental agent GW-9662. They are also used in treating hyperlipidaemia in atherosclerosis. Here they act by increasing the expression of ABCA1, which transports extra-hepatic cholesterol into HDL. Increased uptake and excretion from the liver therefore follows.

Dual-PPAR modulators- A fourth class of "dual", "balanced" or "pan" PPAR ligands, which bind two or more PPAR isoforms, are currently under active investigation for treatment of a larger subset of the symptoms of the metabolic syndrome. These include the experimental compounds aleglitazar, muraglitazar and tesaglitazar. In addition, there is continuing research and development of new PPAR modulators for additional therapeutic indications[8,9].

How do PPARs work at the molecular level?

PPARs possess the canonical domain structure of other NR super family members. The functional domains of the PPARs consist of poorly characterized N-terminal region that contain a potential trans-activation function known as activation factor1 (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 2, 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 AGGNCA half sites 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 heterodimers. 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.

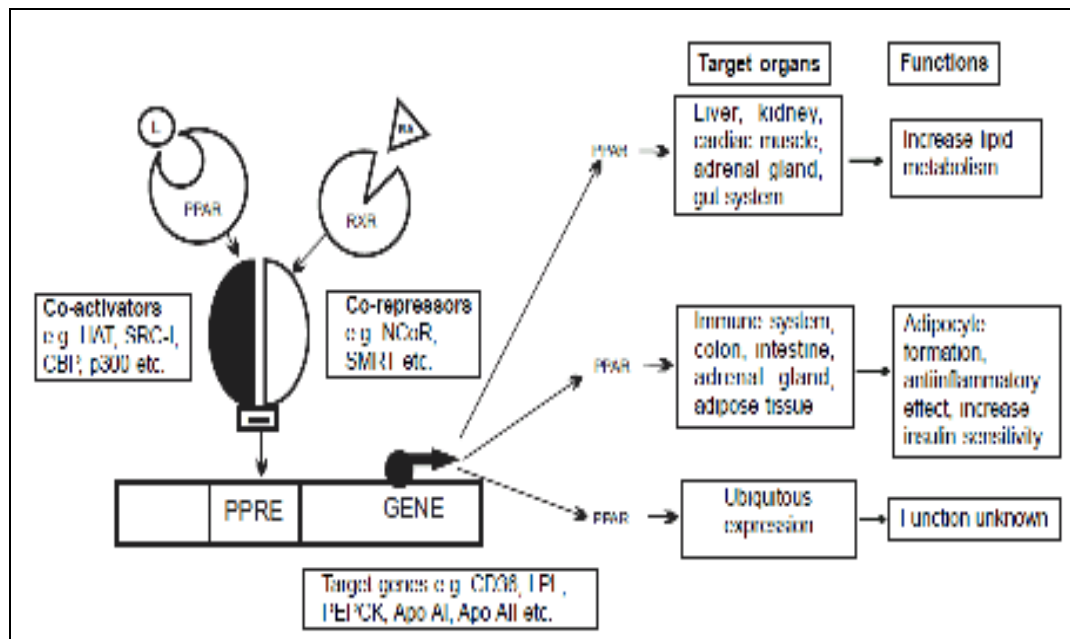


Figure-2 The peroxisome proliferators-activated receptors (PPARs) as transcription factor. As members of the steroid hormone nuclear receptor family, PPARs are thought to control gene expression through a heterodimeric complex with the retinoid X nuclear receptor (RXR). Both PPAR and RXR activation are controlled by binding to specific ligands. The ultimate transcriptional response is determined by the association or release of specific co-activators and co repressors. 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-1= Steroid receptor co-activator, LPL=Lipoprotein lipase, PEPCCK=Phosphoenolpyruvate carboxykinase).

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 heterodimers, 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. Protein with histone acetylase activity which remodels chromatin structure (e.g. SRC-I, CBP). Members of the DRIP/TRAP complex which interact with basal transcription architecture (e.g. PBP/TRAP220). Proteins with incompletely defined function (e.g. PGCI, RIP 140). There are no known receptor specific co-activators or corepressors, 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. 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 [10-14].

Table-1 Exogenous ligands of PPARs:

Compound	Status
PPAR α agonists	
Clofibrate	Marketed
Fenofibrate	Marketed
Bezafibrate	Marketed
Gemfibrozil	Marketed
WY 14643 (Wyeth Pharmaceuticals)	Preclinical stage
GW 7647 (Glaxo Smith Kline)	Preclinical stage
GW 9578 (Glaxo Smith Kline)	Preclinical stage
LY 518674 (Eli Lilly & Co.)	Preclinical stage

PPAR γ agonist

Rosiglitazone (Glaxo Smith Kline)	Marketed
Pioglitazone (Kyorin Pharmaceuticals)	Marketed
KRP-297 (Kyorin Pharmaceuticals, Merck)	Phase I
GW 1929 (Glaxo Smith Kline)	Preclinical stage
GW 7845 (Glaxo Smith Kline)	Preclinical stage
L-165041 (Eli Lilly & Co.)	Preclinical stage
Ciglitazone	Withdrawn from Market.
Troglitazone (Glaxo Smith Kline)	Withdrawn from Market.
TT-501 (Japan Tobacco Inc.)	Terminated

PPAR γ antagonist

MCC 555 (Mitsubishi, J & J)	Phase II
GW 9662 (Glaxo Smith Kline)	Preclinical stage
T 0070907 (Tocris, Sankyo, Tularis)	Preclinical stage
LG 100641 (Ligand Corporation)	Preclinical stage
NC 2100 (Nippon Chemiphar)	Preclinical stage
GW 0072 (Glaxo Smith Kline)	Preclinical stage
BADGE	Investigational tool
PD 068235 (Pfizer Global R & D)	Investigational tool
CDDO-Me (Glaxo Wellcome R & D)	Investigational tool

PPAR δ ligands

GW 501516 (Glaxo Smith Kline)	Preclinical stage
GW 0742 (Glaxo Smith Kline)	Preclinical stage
L-165041 (Merck)	Preclinical stage

PPAR α and γ agonist (Dual PPAR agonists)

Muraglitazar (Bristol Mayer, Merck)	Phase III
Tesaglitazar (Galida) (AstraZeneca)	Phase III
LY 929 (Eli Lilly & Co., Ligand Corporation)	Phase I
LSN 862 (Eli Lilly & Co.)	Preclinical Stage
Ragaglitazar (Dr. Reddy's Lab.)	Terminated

PPAR α , γ and δ (Pan) Agonist

GW 677954 (Glaxo Smith Kline)

Phase II

PLX 204 (Plexxikon Corp)

Phase I

DRL-11605 (Perlecan Pharma)

Phase I

Pharmacological role of PPARs agonists in human disease-**Role of PPAR α agonist-**

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. 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. More recent thioisobutyric acid compounds (GW 7647, GW 9578) show excellent selectivity for PPAR α receptors. Recently reported LY518674 is a novel selective PPAR α agonist [15].

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) 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 .

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

- 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.
- 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.
- Increased removal of LDL particles: Fibrate treatment results in the formation of LDLc with a higher affinity for the LDL receptor, which is thus catabolised more rapidly.
- Reduction in neutral lipid (cholesteryl ester and triglyceride) exchange between VLDL and HDLc may result from decreased plasma levels of TRL.
- 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 [16, 17].

Although fibrates were identified as ligands for the PPAR α receptor, they only show weak agonist activity (high concentrations are required to activate PPAR α) and moderate subtype selectivity in cell-based assays. Consequently, in humans a very high dose (about 300-1200 mg/kg) of these compounds was needed to achieve the desired lipid lowering effect. To provide a superior clinical profile for therapeutic intervention in dyslipidemia and other metabolic disorders more potent and subtype-selective human PPAR α agonists with at least 100 fold selectivity over the other isoforms (PPAR γ and δ) have been reported [18]. In another study the findings that OEA, a naturally occurring fatty-acid ethanol amide, induces satiety and inhibits body-weight gain in rats and mice through activation of the nuclear receptor PPAR- α . The results also suggest that OEA lowers lipid levels in liver and blood, raising the possibility that this compound may exert its anti-obesity effects, at least in part, by enhancing lipid oxidation. Though still speculative, this idea is supported by three lines of evidence. First, OEA stimulates fatty-acid oxidation in isolated muscle, heart and liver cells of lean rats and mice. Second, pair feeding experiments in rats show that hypophagia alone cannot account for the reduction in body-weight gain produced by OEA. Finally, OEA promotes the expression of genes encoding for proteins that are thought to be involved in lipid catabolism and energy balance in lean rats and Zucker rats (present study) [19,20].

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 down regulated by PPAR α agonist in endothelial cells studies. 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). Additionally, PPAR α ligands induce apoptosis of macrophages activated with TNF α or γ Interferon [21]. PPAR- α agonists such as fenofibrate, apart from lowering serum triglycerides and LDL cholesterol and raising HDL cholesterol, improve vasodilator function by up regulating eNOS expression

mainly through mechanisms stabilizing eNOS mRNA. This is a new observation to explain one of the mechanisms of PPAR- α mediated cardiovascular protection [22]. The anti-inflammatory action of PPAR- α on all vascular cells has been studied and reported on, and most notably by both in vitro and in vivo studies. PPAR- α activators inhibit the production of inflammatory response markers such as endothelin-1, vascular adhesion molecule-1 (VCAM-1), interleukin (IL)-6 and tissue factors in endothelial cells, smooth muscle cells and macrophages. In patients with dyslipidemia, PPAR- α agonists reduce the levels of inflammatory markers such as IL-6, fibronogen, C-reactive protein, serum amyloid A, plasminogen, α 2-macroglobin, interferon- γ , IL-2, tumor necrosis factor- α and IL-1 β . These effects of PPAR- α agonists on the vessel wall may explain their cardiovascular protective effects that extend beyond their lipid lowering effect [23]. Additional evidence also suggests that selective activation of PPAR α and PPAR- γ promotes a robust angiogenic process in vitro and in vivo; through a mechanism that depends on the stimulation of the prototypical angiogenic agent vascular endothelial growth factor (VEGF) [24]. Other data from our group also demonstrate that the ability of PGI2 analogues to induce angiogenesis depends on the presence and proper function of the PPAR α gene [25].

Obesity-

Obesity is a risk factor in the development of diabetes and fibrate treatment has been reported to reduce weight gain in rodents. Bezafibrate, Wy-14643 and other agents induce genes involved in increased energy expenditure of fatty acid catabolism [26]. Peroxisome proliferator activated receptor (PPAR) activity may also affect body weight. PPARs are nuclear receptors involved in fat and glucose metabolism. PPAR α receptors are preferentially found in the liver and have historically been the targets of lipid-altering drugs (fibrates), whereas PPAR γ receptors are predominantly found in adipose tissue and have historically been the targets of type 2 diabetes treatments (thiazolidinediones). However, this functional delineation of nuclear receptor types may not be so distinct. Animal studies have suggested that non-PPAR γ agonists (i.e., PPAR agonists without γ activity, such as PPAR α and δ agents) may also result in increased insulin sensitivity and weight loss [27, 28].

Diabetes-

Given that these agents have exhibited improved insulin action and glucose utilization 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 [29]. Another study has been suggested that the agent ANGPTL4 could exert distinct effects on lipid and glucose metabolism mainly through PPAR signaling but not through LXR, because ANGPTL4 mRNA was up-regulated by PPAR α , PPAR γ , and PPAR β/δ agonists. PPAR α plays an important role during fasting via the ligand-dependent transcriptional activation of target genes, while PPAR γ regulates systemic insulin signaling. However, the exact roles of ANGPTL4 in regard to physiology and pathology in humans remain uncertain [30]. PPAR α agonists such as fibrates are not effective hypoglycemic agents, but lowers LDL cholesterol and triglycerides and raise HDL, thus offering protection against increased coronary morbidity and mortality which is seen in type 2 diabetes [31].

Role of PPAR γ agonist-

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. 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. 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 generalized fatty acid sensor that couples changes in overall PUFAs' concentration with the target genes associated with lipid and glucose homeostasis. 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 sensitizing properties while minimizing potential adverse effects. GW 0072 (non TZD thiazolidine acetamide) antagonizes the adipocyte differentiation induced by rosiglitazone but promotes adipocyte differentiation in the presence of insulin and, hence, functions as insulin sensitiser. 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].

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. TZDs are the new class of drugs useful in the treatment of type-2 diabetes. Recent advances include the discovery of novel 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?" 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 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. 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. Over expression 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 [34, 35].

In general, pioglitazone and rosiglitazone have similar clinical efficacy with both demonstrating improvements in insulin sensitivity and the ability to lower fasting plasma glucose levels. TZD is the greater impact of pioglitazone on diabetic dyslipidemia. Although pioglitazone was initially found to be a PPAR γ agonist, additional preclinical data show that this compound has some, although minimal, activity on PPAR γ as well in standard cotransfection (CTF) assays. In addition to the improvement in glycemic control, the glitazones have a beneficial effect on many of the traditional as well as the new risk factors and can help in preventing or lessening the impact of the cardiovascular consequences of type 2 diabetes. They have been shown to lower the levels of atherogenic dyslipidemia, lower blood pressure as well as visceral obesity, lessen the levels of the pro-inflammatory and pro-thrombotic cytokines and adipokines as well as increase the levels of the antiatherogenic adinopectin [36]. They decrease hepatic glucose output and increase peripheral glucose utilization by improving insulin sensitivity at hepatic and muscle sites. They restore the sensitivity of phosphophenol pyruvate carboxy kinase (PEPCK) to insulin thereby decreasing glycogenolysis. They also increase peripheral triglyceride clearance and decrease hepatic triglyceride synthesis, independent of insulin. At the cellular level, they increase the binding and tyrosine kinase activity of insulin receptors, activate post receptor signaling

proteins and enhance insulin induced translocation of GLUT-4 on to the plasma membranes. All these effects are dependent on insulin. These agents do not stimulate insulin secretion from β -cells and are therefore not effective in insulinopenic subjects. A new class of drugs which are plant extracts and act through inhibition of protein tyrosine kinase are being investigated. In addition to hypoglycemic effect it blocks the formation of proinflammatory cytokines such as TNF α . Compounds in this class includes CLX-0301, CLX 0302, CLX 0900, and CLX 0901. This group of drugs also lowers cholesterol and triglycerides. Again these are sensitizers and are not effective in type 1 diabetes [37].

Another study has been find out the relation between Pro 12 Ala polymorphism of PPAR γ 2 gene with type 2 diabetes mellitus and the possible role of this gene polymorphism as a link between obesity and type 2 diabetes mellitus by using polymerase chain reaction. Significant increased in Pro allele was found in diabetic non obese patients & diabetic non obese patients when compared to control group (p= 0.048 and 0.003, respectively). However, there was no difference between group obese non diabetic and control group as regard the allelic frequencies (p= 0.462). Activation of PPAR γ 2 results in an increase in the sensitivity of both the liver to insulin-mediated suppression of hepatic glucose production and insulin mediated skeletal muscle glucose uptake [38]. PPAR α and PPAR γ have been reported to up regulate nitric oxide synthase (NOS) and stimulate NO release, which is beneficial to counteract endothelial dysfunction commonly present in patients with metabolic syndrome especially obesity, insulin resistance and type 2 diabetes [39].

Atherosclerosis-

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* [40] 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 [41, 42]. In addition, previous clinical data

indicates that PPAR γ agonist (troglitazone) actually protects type-2 diabetic patients from atherosclerosis.

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 stabilization and regression.

Thus, TZDs show potential as potent anti-inflammatory, antithrombotic agents which could improve glucose tolerance and the long-term cardiovascular risk related to atherosclerosis in patients with type-2 diabetes [43].

Another study has been reported that the effect of fenofibrate treatment on atherosclerosis was assessed in E2-KI mice by measuring oil-red-O–stained surfaces at the aortic sinus. Fenofibrate-treated mice showed significantly decreased atherosclerotic lesion surfaces (-90%) when compared with control mice (0.0140 ± 0.0123 versus 0.0719 ± 0.0189 mm²; $P \leq 0.001$). In marked contrast, rosiglitazone and pioglitazone had no effect on atherosclerotic lesion size (0.0666 ± 0.0229 and 0.0626 ± 0.0257 mm², respectively). However, in both previous studies, it seemed that PPAR α ligands exert their antiatherosclerotic effects via mechanisms unrelated to their plasma lipid-lowering activity. This observation is in line with a number of clinical studies reporting that the inhibition of atheromatous plaque progression as well as the decreased incidence of cardiovascular events on fibrate treatment occurs via effects possibly independent or in addition to their systemic action. Atherosclerosis in the E2-KI mice, in contrast to other mouse models, is characterized by the almost exclusive presence of macrophages. It is possible that in the context of severe, uncorrected dyslipidemia, PPAR γ activation in macrophages is insufficient to reverse the pro-atherogenic program. Moreover, a substantial controversy exists on the role of PPAR γ in macrophage cholesterol metabolism in mice. In vitro, PPAR γ ligands may induce macrophage foam cell formation through CD36 induction and increased uptake of oxidized LDL [44].

Inflammation-

Several investigators have established PPAR γ expression in monocytes/macrophages and human atherosclerotic lesions [45, 46]. Jiang *et al* [47] 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 monocytes like cell lines. Ricote *et al* [48] 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 anti-inflammatory agent in animal models of acute inflammation. In vitro reports find PPAR γ inhibition of monocytes chemo attractant 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 [29]. PPAR γ agonists have also been reported by Pasceri *et al* [49], to inhibit other macrophage proinflammatory mediators, including iNOS, gelatinase B, and the macrophage scavenger receptor-A. PPAR- γ activation suppresses gastric mucosal inflammatory responses to *Helicobacter pylori* (*H pylori*) LPSs, suggesting that pharmacological manipulation of PPAR- γ activation may provide therapeutic benefits in the resolution of inflammation associated with *H pylori* infection. The mechanism of PPAR- γ and its ligand to regulate cellular inflammation may involve multiple pathways in different kinds of cells and the state of differentiation/ activation of the same source of cells. In macrophages and epithelial cells, the effect of 15 d-PGJ2 is targeted to the NF- κ B/I- κ B pathway and to the mitogen-activated protein kinase ERK1/2. The role of PPAR- γ activation in tissue factor inhibition by 15 d-PGJ2 is excluded. 15 d-PGJ2 and rosiglitazone rapidly induce the transcription of suppressor of cytokine signalings 1 and 3, which in turn inhibit Janus kinase (JAK) activity in activated glial cells. In addition, Src homology 2 domain-containing protein phosphatase 2, another negative regulator of JAK activity, is also involved in their anti-inflammatory action. Although it is not a direct causal effect, the insufficient PPAR- γ activity contributes to ongoing dysregulated inflammation in pulmonary sarcoidosis by failing to suppress NF- κ B [50]. In another study has been find out 3 weeks of CG administration yielded EPS characterized by loss of UF, increased peritoneal thickness, inflammation, destruction of peritoneal membrane integrity, and ultimately, the development of fibrosis and

encapsulation. Peritoneal rest provides some advantages, but only with regard to UF failure, D/P urea, and dialysate cell count. But the PPAR gamma agonist rosiglitazone was more effective than rest for almost all of the structural and functional parameters of peritoneum considered in the present study. With rosiglitazone, observed improvements in all functional and morphologic parameters of the peritoneum exposed to CG. Although the present model is more suitable for an examination of morphologic parameters than of functional parameters, an improvement in D/P urea and UF capacity with rosiglitazone. The decreased inflammatory cell count and neoangiogenesis might ultimately lead to less thickness and increased UF capacity. Use of PPAR gamma agonists to regulate TGF/Smad pathways in peritoneum after exposure to PD solutions had been shown to inhibit well-known fibrotic pathways. That TGF/Smad pathway may play a role in the beneficial results seen in the present study [51].

Cancer-

PPAR γ is highly expressed in several human cancer cell lines, including liposarcoma [52], breasts [53], colon [54], lungs [55], prostate [56], bladder and gastric [57, 58]. 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 tumor growth suppression in vivo rodent carcinoma models. Promising phase 2 clinical trials, with troglitazone, suggest that the use of these agents offer an improved therapeutic treatment opportunity for inoperable lipocarcinomas [59]. Another study demonstrates that the structurally distinct, synthetic PPAR γ ligands PIO, GW, and ciglitazone inhibit proliferative responses and proinflammatory cytokine secretion of mitogen-stimulated PBMCs derived from MS patients and HDs. Additionally, antiproliferative effects were demonstrated in Jurkat cells and the TT-specific T cell line KHS.TT2. Inhibitions of proliferation as well as IFN γ and TNF α secretion were most pronounced after preincubation of PBMCs with PIO and GW, suggesting a sensitizing effect of these drugs. In support of this assumption, the antiproliferative effects of PIO in vitro were increased in one MS patient treated with PIO (15 mg tid) for 2 months. The induction of apoptosis seems to contribute to the antiproliferative and anti-inflammatory action of PPAR γ agonists in vitro. PPAR γ agonists exert their immunomodulatory effects, at least in part, by interfering with the activation of T cells [60].

Role of PPAR- δ agonist-

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, adipogenesis, colon cancer, bone metabolism, embryo implantation and development of brain and skin [61, 62,].

Lipid metabolism-

PPAR δ selective ligands have been reported to increase HDLc in diabetic db/ db mice without effects on blood glucose and triglyceride concentration. 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 [63]. Significantly, GW501516 raised HDLc levels more than fenofibrate in this model. PPAR δ has also exhibited a potential role in placentation, adiposity, colorectal cancer, and diabetic factors. GW0742 is a closely related analog of GW501516 and shows equivalent potency and selectivity for PPAR δ [64].

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 [65].

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 [66].

Other role of PPARs agonists in human disease-***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 [67]. Other studies also demonstrated that ligands of the PPAR γ induce apoptosis in activated T-lymphocytes and exert anti-inflammatory 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* [68] 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. *In vitro* studies have demonstrated that PPAR γ agonists modulate inflammatory responses to bacterial endotoxin in brain and also prevent endotoxin-induced neuronal death [69]. Peroxisome proliferator-activated receptor agonist mediated preservation of insulin/IGF responsive neurons was associated with increased expression of ChAT, which has an important role in cognition, as cholinergic neuron deficits are a fundamental feature of AD. Importantly, the PPAR- δ agonist mediated increases in insulin binding, and ChAT were associated with significant improvements in learning and spatial memory tasks as demonstrated using Morris water maze tests. These effects of the PPAR agonist treatments are consistent with the facts that ChAT expression is regulated by insulin/IGF and insulin/IGF resistance mediates cognitive impairment in AD. The PPAR-mediated increases in MAG-1 expression, corresponding to oligodendroglia, were of particular interest because previous research demonstrated that one of the earliest AD lesions was white matter atrophy and degeneration with loss of oligodendroglial cells [70].

Role of PPARs in hair growth-

An interesting investigation found that the expression pattern of the PPAR α , δ and γ subtypes and their role in human hair follicles. Using reverse transcriptase-polymerase

chain reaction and immunohistochemistry, established that PPAR α , - δ and - γ were expressed in both dermal and epithelial human hair follicle cells. Additionally, evaluated the dose effect of clofibrate, a PPAR α ligand, on the survival of human hair follicles in culture. A beneficial effect was observed within a narrow range of concentrations. Taken together these clinical studies clearly demonstrate that an unbalanced lipid metabolism can lead to an alteration of the human hair cycle [71].

Role of PPARs in CVS-

PPAR α activation increases the expression of lipoprotein lipase and apolipoprotein A-V (apoA-V) while simultaneously decreasing expression of apoC-III in the liver, which decreases VLDL particles and lowering plasma triglycerides. Thus, PPAR α agonists may therefore be beneficial in aiding fat loss. PPAR γ is involved in the storage of fatty acids and its activation can either increase or decrease the transcription of genes and enzymes involved in insulin sensitivity and adipogenesis, adipocyte differentiation, cell proliferation, and the inflammatory process. All of these effects play a role in obesity and metabolic syndrome X (abdominal obesity, insulin resistance, and elevated blood pressure) [72].

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. These dual-acting PPAR agonists are a novel group of compounds which also activate nuclear transcription factors. The examples are muraglitazar, farglitazar, ragaglitazar, reglitazar, tesaglitazar, LSN 862, LY 929 and so on [73]. 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 [29]. 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 [74]. Ragaglitazar exhibits better plasma glucose and triglyceride reduction than rosiglitazone in insulin resistant db/db mice [75]. 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 [76]. BMS-298585 is an oxybenzylglycine with potent and selective, balanced PPAR α and γ agonist effects [29]. 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 [76].

The old and well-known lipid-lowering fibric acid derivative bezafibrate is the first clinically tested pan (α , β/δ and γ) PPAR activator. 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 [77]. Recently, GSK and Plexxikon Inc. discovered the novel pan agonists, GW 67795 and PLX 204 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 [78] 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. Preclinical study revealed improved insulin sensitivity and alleviated diabetic hyperglycemia with less weight gain with

administration of this dual PPAR γ/δ agonist in comparison to rosiglitazone [79]. This puts development of other glitazars by such companies as Lilly, Ligand, Roche, Mitsubishi, and Novartis, as well as PPAR pan-agonists (i.e., alpha, beta, and gamma agonists), which are being developed by GlaxoSmithKline and Plexxicon, under a dark cloud [80].

Toxicity induced by PPAR α agonists-

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 [81]. Dr. Reddy's ragaglitazar, when studied for long-term rodent toxicity, revealed that it was associated with bladder cancer. 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 [82]. 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 humanized mouse line was generated in which the human PPAR-alpha was expressed in liver under control of tetracycline responsive regulatory system. The PPAR-alpha humanize 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-humanized mice exhibited hepatocellular proliferation, as revealed by elevation of cell cycle control genes and hepatomegaly. We need to identify novel ways to modulate PPAR γ activity without complicating issues such as the enhancement of macrophage foam cell formation, stimulation of colon carcinogenesis and induction of acute liver dysfunction [84]. 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 proximal and/or lipid homeostasis gene expressions 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 [85].

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. The potential impact PPAR ligands on metabolic syndrome, the associated cardiovascular risk factors, type 2-

diabetes have made research of PPARs attractive. In conclusion, though the full therapeutic potential of PPARs has yet to be realized, and serious safety issues are associated with the currently marketed PPAR drugs (PPAR- α and PPAR- γ), there remains intense interest in exploring new physiological roles of the PPARs and in the identification of new and improved PPAR agonists drugs. Therefore, understanding how the PPAR genes and other hormone nuclear receptors are regulated during disease processes will provide us the opportunity to design effective therapeutic modalities to treat disease by the inactivation, conjugation, and transport of toxic endogenous metabolites.

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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