Peroxisome proliferator-activated receptor- δ , a regulator of oxidative capacity, fuel switching and cholesterol transport

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Abstract Synthetic agonists of peroxisome proliferator-activated receptor (PPAR)- δ have shown a promising pharmacological profile in preclinical models of metabolic and cardiovascular disease. At present, the pharmaceutical development of these drugs exploits the potential to raise plasma HDL-cholesterol in animals and their insulin-sensitising and glucose-lowering properties. PPAR- δ agonists have also proven to be powerful research tools that have provided insights into the role of fatty acid metabolism in human physiology and disease. Activation of PPAR- δ induces the expression of genes important for cellular fatty acid combustion and an associated increase in whole-body lipid dissipation. The predominant target tissue in this regard is skeletal muscle, in which PPAR- δ activation regulates the oxidative capacity of the mitochondrial apparatus, switches fuel preference from glucose to fatty acids, and reduces triacylglycerol storage. These changes counter the characteristic derangements of insulin-resistant skeletal muscle but resemble the metabolic adaptation to regular physical exercise. Apart from effects on fuel turnover, there is evidence for direct antiatherogenic properties, because PPAR- δ activation increases cholesterol export and represses inflammatory gene expression in macrophages and atherosclerotic lesions. Whereas conclusions about the full potential of PPAR- δ as a drug target await the result of large scale clinical testing, ongoing investigation of this nuclear receptor has greatly improved our knowledge of the physiological regulation of whole-

body fuel turnover and the interdependence of mitochondrial function and insulin sensitivity.

Keywords Atherogenesis · HDL-cholesterol · Insulin sensitivity · Peroxisome proliferator-activated receptor · Pharmacology · PPAR- δ · Receptor agonist · Skeletal muscle

Abbreviations

Apo	apolipoprotein
BAT	brown adipose tissue
PPAR	peroxisome proliferator-activated receptor
PGC-1 α	PPAR- γ coactivator-1 α
RXR	retinoic X receptor
UCP-1	uncoupling protein 1
WAT	white adipose tissue

Introduction

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors with pleiotropic biological functions that include regulatory roles in lipid metabolism and whole-body fuel turnover. The extraordinary interest in this family of nuclear receptors relates to the observation that two out of the three described subtypes are molecular targets of drugs used for the treatment of prevalent metabolic disorders. The triacylglycerol-lowering fibrates are agonists of PPAR- α , while the insulin-sensitising glitazones activate PPAR- γ and are prescribed for the treatment of type 2 diabetes [1, 2]. The therapeutic potential of PPAR- α and PPAR- γ has fuelled efforts to understand their biological functions and the molecular mechanisms of action of synthetic agonists. PPAR- δ (also referred to as PPAR- β) initially attracted less attention owing to the lack of synthetic agonists and ubiquitous expression of this subtype

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in almost all tissues of the body [3–5]. Nevertheless, evidence from PPAR- δ knock-out mice and other experimental approaches revealed a broad spectrum of functions in embryo implantation, early development, wound healing and cancer development [6–9]. The important regulatory impact of PPAR- δ on whole-body fuel turnover was not recognised until potent and selective agonists became available [10–13]. The subsequent pharmacological studies promoted great interest in this receptor as a target for drug development [10, 13–17]. Investigations have focused on PPAR- δ agonists following evidence that they regulate signalling pathways regarded as fundamental and causal in the development of insulin resistance and atherosclerosis. This review presents our current knowledge of the regulatory role of PPAR- δ in glucose and lipid metabolism and highlights the potential of the receptor as a target for the treatment of dyslipidaemia, insulin resistance and atherosclerosis.

Evidence for therapeutic potential

Improvement of the plasma lipid profile

The evidence that activation of PPAR- δ can ameliorate derangements of the plasma lipid profile is abundant and unanimous. Since lipid metabolism differs considerably between rodents and humans, it is important that these effects are documented in mice [13, 14, 16, 18] as well as in monkeys [10, 17]. In both non-human primate and mouse models of dyslipidaemia, PPAR- δ agonists decrease circulating NEFA, triacylglycerol and ketones [10, 14, 16]. However, their most powerful effect is on plasma HDL-cholesterol, which increases along with the two major lipoprotein components of HDL particles, apolipoprotein (Apo) AI and AII [10, 13, 17–19]. In rhesus monkeys with lipid derangements resembling those of obese prediabetic humans, the PPAR- δ agonist GW501516 more than doubled circulating HDL-cholesterol while reducing plasma triacylglycerol, insulin, VLDL-triacylglycerol and LDL-cholesterol, as well as shifting the LDL subspecies towards a larger particle size [10]. Marked increases in plasma HDL-cholesterol were also found in agonist-treated vervet monkeys and mice that did not show any changes in triacylglycerol, glucose or insulin, which further illustrates that an increase in HDL-cholesterol is the predominant consequence of PPAR- δ activation [13, 17–19]. A similar role of PPAR- δ in human lipid homeostasis is suggested indirectly by the association of a polymorphism in the gene encoding PPAR- δ with differences in cholesterol metabolism and plasma HDL [20, 21], and by initial clinical data on the HDL-raising properties of GW501516 in healthy volunteers [22].

PPAR- δ agonists thus induce changes in the lipid profile that are believed to be associated with reduced atherosclerosis and a reduced risk of coronary heart disease [23, 24]. Consequently, present efforts in drug discovery have targeted the development of PPAR- δ agonists for atherogenic dyslipidaemia with the focus on their potential to raise HDL. The possible value of this treatment option is underlined by the fact that widely prescribed statin and fibrate drugs are not very effective in raising HDL. The superiority of PPAR- δ agonists over established drugs is exemplified by a study on vervet monkeys, in which GW501516 was twice as effective in raising HDL-cholesterol as the PPAR- α agonist fenofibrate [17].

PPAR- δ agonists improve the lipid profile and are particularly potent in raising HDL-cholesterol.

Insulin sensitisation and antihyperglycaemic action

Hard data that directly document antihyperglycaemic action of PPAR- δ agonists stem from mice subjected to regular oral administration of GW501516 [14, 15, 19]. In mice developing obesity as a result of a high-fat diet, GW501516 improved glucose tolerance with a parallel reduction in circulating insulin. In genetically obese mice afflicted with more severe hyperglycaemia, basal glucose and insulin were also reduced, but the insulin response to a glucose stimulus was increased, hinting at recovery of exhausted pancreatic beta cells. Insulin tolerance tests and euglycaemic–hyperinsulinaemic clamp tests showed that PPAR- δ -dependent glucose-lowering was related to whole-body insulin sensitisation [14, 15, 19]. Treatment of healthy mice with GW501516 also blunted glucose and insulin excursions in the glucose tolerance test, but, importantly, it did not affect basal glucose in normoglycaemic mice or monkeys [10, 14]. Such antihyperglycaemic potential without any hypoglycaemic action suggests that insulin sensitisation is the cause rather than the consequence of glucose-lowering and that PPAR- δ agonists are to be categorised as true insulin sensitisers.

Although the insulin-sensitising properties of the PPAR- δ agonists are undisputed, their glucose-lowering actions fall quantitatively behind those of the PPAR- γ -agonists. As a consequence, selective PPAR- δ agonists have been developed as HDL-raising rather than antihyperglycaemic agents. While these comparisons await to be confirmed in humans, a modest insulin-sensitising activity would be a desirable attribute of an agent administered to patients with atherogenic dyslipidaemia.

PPAR- δ agonists are insulin sensitisers, but their glucose-lowering potential seems to be less than that of their sister drugs, the glitazones.

Mechanisms of action

Initial molecular events

As extensively summarised in other reviews [1, 25], the early molecular events following the activation of PPAR- δ are well established and resemble those of the other PPAR subtypes. In short, the natural ligands of PPAR- δ are fatty acids and their metabolites, with a superior efficacy attributed to unsaturated fatty acids and specific eicosanoids [1, 25–28]. Since the PPARs form obligatory heterodimers with retinoic X receptor- α (RXR- δ), ligands of the two partner receptors can act in a synergic manner [1, 29, 30]. The activity of this receptor complex is further controlled by stimulatory and inhibitory cofactors that are recruited and released in a ligand-specific manner. The final composition and action of the receptor–ligand–cofactor complex is influenced not only by the individual ligand, but also by cofactor availability within the specific tissue and the prevailing physiological or pathophysiological environment. The resulting protein complex directly modulates gene expression by interaction with peroxisome proliferator response elements located in gene promoters, as well as via interaction with other transcription factors. For all PPAR subtypes, the genes regulated in this way are predominantly involved in cellular transport, storage, and metabolism of lipids [1, 31], which, with the typical delay of genomically mediated actions, ultimately translates into a metabolic response.

Upon activation, PPAR- δ interacts with cofactors and modulates the expression of target genes.

Whole-body fat dissipation

Increased fat burning and energy expenditure Many of the genes regulated by PPAR- δ are implicated in the transport, activation, and β -oxidation of fatty acids, in energy uncoupling and dissipation, and in mitochondrial biogenesis [14, 29, 32–35]. As predicted from this biochemical pathway, the most pronounced metabolic effect of PPAR- δ activation is an increase in the oxidation and dissipation of lipids. Stimulation of fat burning explains why PPAR- δ activation is particularly effective in protecting against the consequences of dietary lipid overload [14–16]. In high-fat-fed mice, GW501516 markedly blunted weight gain in spite of unchanged food intake and induced an approximately 25% increase in resting O_2 consumption [14, 15]. These data imply that PPAR- δ activation leads to an increase in energy expenditure, albeit a contributory role of reduced intestinal fat absorption cannot be ruled out (as reported for cholesterol [18]). In line with a specific increase in fat dissipation, weight loss in the fat-fed mice was due to lower

adipose tissue mass together with reduced lipid accumulation in muscle and liver [14, 15].

Hence, PPAR- δ activation can reduce lipid stores in muscle, liver and fat, which are the most important tissues for whole-body fat burning and insulin-dependent glucose homeostasis. When these tissues were collected from GW501516-treated mice, fatty acid oxidation ex vivo was elevated by approximately 50% in skeletal muscle, whereas it had increased only modestly in brown adipose tissue (BAT) and remained unchanged in the liver [14]. Blunted hepatic fat accumulation under PPAR- δ stimulation therefore seems to relate to changes in whole-body lipid balance rather than to local combustion. Accordingly, hepatic lipid content was also reduced in mice subjected to tissue-specific over-activation of PPAR- δ in muscle or fat only [15, 16, 36].

Tissue-specific roles of PPAR- δ and PPAR- α Different sensitivities of muscle and liver to PPAR- δ activation are of interest with regard to the question of why agonists of PPAR- δ and PPAR- α exhibit a different pharmacology, even though they activate transcription of a largely overlapping set of genes [32, 37, 38]. Both receptor subtypes are expressed to at least some extent in skeletal muscle as well as in liver [3, 4, 37], but their efficacy in stimulating fat-burning appears to be tissue-specific. In contrast to GW501516, the PPAR- α agonist fenofibrate markedly increased fatty acid oxidation ex vivo by the liver, but not by skeletal muscle [14]. Absence of a major role for PPAR- α in skeletal muscle is further corroborated by the finding that isolated rat muscle is much more sensitive to a PPAR- δ - than a PPAR- α -specific agonist [39], and by ablation of PPAR- α having little influence on fuel metabolism of skeletal muscle [37]. It should be noted that such tissue specificity is not necessarily maintained in cultured cell lines [37, 38], but is consistent with the proposed physiological roles of PPAR- α as a mediator of fasting-induced hepatic ketone production and of PPAR- δ as a mediator of muscle adaptation to exercise [15, 36, 40].

PPAR- δ activation increases whole-body fat-burning and energy dissipation. Compared with PPAR- α the pharmacology of the PPAR- δ subtype is more important in muscle and less important in the liver.

Oxidative capacity and fuel switching in skeletal muscle

PPAR- δ activation boosts mitochondrial capacity in skeletal muscle Because of the broad expression of the gene encoding PPAR- δ [3–5], many tissues and cells could contribute to increased whole-body lipid combustion, but the dominant pharmacology can clearly be attributed to

skeletal muscle. The action of PPAR- δ agonists on muscle is direct, since the increases in PPAR- δ target gene expression and fatty acid oxidation triggered in cell lines and tissue specimens in vitro resemble the response seen in skeletal muscle from orally dosed animals [14, 16, 29, 34, 35, 37, 41]. In this context, it is of note that the induced genes include many encoding mitochondrial proteins and perhaps also that for PPAR- γ coactivator-1 α (PGC-1 α) [14, 29], a key stimulator of the mitochondrial biogenesis programme [42, 43]. Since PGC-1 α functions as a coactivator that boosts PPAR- δ activation by direct protein–protein interaction [16, 29], stimulation of mitochondrial biogenesis by PGC-1 α could at least in part be PPAR- δ -mediated and could be relevant for the downstream metabolic responses.

It is not yet clear whether the response to PPAR- δ activation includes true mitochondrial biogenesis (i.e. the generation of new organelles) or is restricted to growth and/or structural improvement of pre-existing mitochondria. However, there is evidence of a functional improvement of the mitochondrial apparatus as a whole, as seen by the enriched mitochondrial density in skeletal muscle of agonist-treated mice [14]. Although genetically manipulated models allow only limited conclusions about normal physiology, the impact on the mitochondrial apparatus has been pinpointed impressively by targeted overexpression of PPAR- δ or a constitutively active form of PPAR- δ in skeletal muscle [15, 36]. Muscle from transgenic mice was composed of a larger proportion of red muscle fibres characterised by high oxidative capacity, although it remains to be determined whether these phenotypic changes fulfilled all criteria of true fibre-type conversion from type 2 to type 1. Modulation of fibre characteristics was associated with protection from the deleterious effects of high-fat feeding, including amelioration of weight gain, fat cell hypertrophy, lipid accumulation in muscle, and glucose intolerance [15, 36]. Similar effects have been ascribed to muscle-specific overexpression of PGC-1 α , which, together with more abundant expression of PPAR- δ and PGC-1 α in red than white fibres, reflects the importance of coordinated expression and interaction of these two proteins [15, 44]. It is of note, however, that reduced expression of the gene encoding PGC-1 α in type 2 diabetes is not paralleled by decreased levels of PPAR- δ , which therefore should remain available as a drug target [45, 46].

PPAR- δ , a mediator of adaptation to regular exercise The effect of PPAR- δ activation in skeletal muscle is highly reminiscent of the adaptive response to regular physical training, which also enhances the levels of PPAR- δ and PGC-1 α , as well as increasing mitochondrial capacity and insulin sensitivity [36, 47–49]. Accordingly, transgenic mice with a constitutively activated form of PPAR- δ in skeletal muscle were capable of running nearly twice as

long and twice as far as their wild-type littermates, without any physical training [15]. It is tempting to speculate that PPAR- δ could be a physiological mediator of adaptation to regular exercise and that PPAR- δ agonists could turn on the same molecular cascade as that activated by endurance training. Considering that the undisputed benefit of regular physical activity on insulin resistance and type 2 diabetes usually remains unexploited, the option to activate these mechanisms in sedentary patients by conventional pharmacological intervention underpins the attractiveness of PPAR- δ as a potential drug target.

PPAR- δ and the glucose–fatty acid cycle An increase in fatty acid oxidation—be it caused by high ambient lipids or PPAR- δ activation—reduces the glucose utilisation of isolated muscle as a result of the mutual inhibition of substrate metabolism in the glucose–fatty acid cycle [39, 50]. It is a broadly accepted paradigm that such a shift in fuel preference will, in the long-term, impair insulin sensitivity and glucose homeostasis in vivo. However, this premise seems to hold true only when rising ambient lipid concentrations ‘push’ substrate into cells not prepared to handle increased lipid influx [50, 51]. This effect is to be distinguished from PPAR- δ activation, where a preceding increase in the capacity for fat dissipation stimulates the muscle cell to ‘pull’ more substrate into the lipid oxidation pathway (Fig. 1). Rather than being a direct consequence of impaired glucose utilisation, lipid-induced insulin resistance is thus due to an imbalance between the requirement and the capacity for fat burning. Indeed, insulin-resistant muscle is characterised by impaired rather than enhanced fatty acid oxidation and a shift in basal fuel selection from fatty acids to glucose [52–54]. Insulin-resistant muscle is composed of relatively fewer oxidative type 1 fibres, and its mitochondria are small, poorly structured and of reduced bioenergetic capacity [55–58]. Intramyocellular lipid accumulation, another hallmark of insulin-resistant muscle [58–60], could thus be the consequence of a disequilibrium between lipid influx and lipid combustion. Since the correlations between insulin sensitivity, mitochondrial function and intramyocellular lipids are strong and prevail even in muscle from healthy groups at increased risk of developing diabetes [59–62], these disturbances are regarded as fundamental in the progressive development of insulin resistance and type 2 diabetes [58, 60, 63]. Piecing the puzzle together, PPAR- δ -induced improvements in oxidative capacity and fat utilisation in skeletal muscle could lead to the reversal of early and causal factors in the development of metabolic disease.

A recent report suggested that direct effects of PPAR- δ activation in the liver could also contribute to the contrary effect of lipid overflow vs PPAR- δ activation on whole-body insulin sensitivity [19]. In agonist-treated mice,

Fig. 1 Increased lipid oxidation in skeletal muscle: 'push' vs 'pull' mechanisms that determine the associated change in insulin sensitivity. *Pink shading* ('push' mechanism): An increase in the ambient lipid concentration 'pushes' more fatty acids into the cell, where the potential to increase mitochondrial lipid oxidation is limited. Insulin resistance develops and intracellular lipid stores accumulate. *Green shading* ('pull' mechanism): Activation of PPAR-δ strengthens the mitochondrial apparatus and the

changes in hepatic gene expression increased glucose flux through the pentose phosphate pathway, enhanced fatty acid synthesis from glucose, and reduced endogenous glucose production [19]. It should be noted, however, that the major contribution of hepatic PPAR-δ to changes in whole-body fuel metabolism observed in this study appears difficult to reconcile with other observations and needs to be confirmed by other laboratories.

PPAR-δ and the myocardium Albeit the contribution of the heart to whole-body fuel turnover is minor, it is notable that PPAR-δ agonists increase the expression of genes encoding proteins involved in lipid oxidation and fatty acid oxidation, not only in skeletal muscle but also in cardiomyocytes [32, 33]. Mice with a cardiomyocyte-restricted deletion of PPAR-δ showed progressive development of cardiomyopathy accompanied by lipid accumulation, disrupted mitochondrial structure, and a shift from fatty acid towards glucose utilisation in the myocardium [64]. These dis-

potential to oxidise lipids. More fatty acids are 'pulled' into the cell and oxidised, intracellular lipid stores are dissipated in association with insulin sensitisation. *Yellow shading*: Independent of the underlying cause, increased lipid oxidation decreases glucose utilisation via the glucose-fatty acid cycle, which can be associated with an increase or a decrease in insulin sensitivity

turbances resemble the characteristics of advanced heart failure [65]. Hence, it appears that similar derangements in fuel handling of the striated muscle cell accompany, or perhaps even underlie, peripheral insulin resistance and cardiac dysfunction, often in the same patient. This relationship suggests that PPAR-δ could be key to understanding the interdependence of mitochondrial capacity, exercise, insulin sensitivity and myocardial function.

Activation of PPAR-δ boosts mitochondrial capacity and lipid oxidation in the striated muscle cell, which resembles the effect of regular exercise and counters derangements associated with insulin resistance and heart failure.

BAT and adipogenesis

Increased fat burning in adipose tissue GW501516 has been shown to elevate fatty acid oxidation by adipocytes in vitro and ex vivo, and adipose tissue-specific overactivation of

PPAR- δ in mice stimulated fat metabolism and prevented obesity [14, 16]. Hence, there is no doubt that activation of PPAR- δ accelerates lipid dissipation, not only in muscle but also in adipose tissue. In mice, lipid burning by adipocytes occurs mainly in BAT, which, in contrast to white adipose tissue (WAT), is rich in mitochondria and has a high oxidative capacity [66, 67]. In high-fat-fed mice, GW501516 increased fatty acid oxidation, reduced cell size and blunted lipid accumulation in BAT [14]. In genetically obese diabetic mice (*db/db*), the PPAR- δ agonist reversed the WAT-like appearance of BAT by enrichment with mitochondria and replacement of a single large lipid droplet per cell with multiple small droplets [16]. Furthermore, PPAR- δ and PGC-1 α can increase expression of the gene encoding uncoupling protein 1 (UCP-1), a BAT-specific mitochondrial protein that channels fuel-derived energy into heat rather than ATP production [16, 68]. In rodents, BAT functions as a flow heater to counteract loss of body temperature, and fat burning by brown adipocytes could contribute significantly to PPAR- δ -induced changes in whole-body fuel turnover. However, heat emission is dependent on body size and, hence, BAT is abundant only in small mammals and virtually absent in adult humans, in whom skeletal muscle is the primary thermogenic organ [66, 67]. Although isolated human WAT cells can acquire certain features of BAT under experimental conditions [68], there is no evidence for conversion of white into brown adipocytes *in vivo*. The low capacity for fuel combustion in WAT, together with the virtual absence of BAT, argue against extensive PPAR- δ -induced lipid dissipation by adipose tissue in humans, in whom skeletal muscle is more likely to drive the pharmacology.

PPAR- δ -induced adipogenesis? There has been speculation that PPAR- δ , like PPAR- γ , could be implicated in regulation of adipogenesis and that the two subtypes could modulate insulin sensitivity via similar mechanisms. PPAR- γ agonists act by interference with a regulatory feedback loop that tightly couples the degree of adiposity to insulin sensitivity. This physiological function may have evolved as a mechanism to manage fuel storage in periods of abundant food supply. Part of the feedback loop is the adiposity-dependent release of fatty acids and peptide hormones (adipokines) from adipocytes, providing a signal that modulates insulin sensitivity in other tissues, such as skeletal muscle and liver [69–71]. Adipogenic PPAR- γ agonists remodel adipose tissue into a more insulin-sensitive form composed of many small adipocytes. Since hormone release is determined by the hypertrophy of the individual fat cell rather than by the total adipose mass, PPAR- γ activation results in an increase in total body insulin sensitivity [72–75]. In contrast, parallel data supporting similar mechanisms of action for PPAR- δ are restricted to relatively artificial experimental settings. *In vitro*, PPAR- δ can stimulate the

early steps of adipogenesis and trigger expression of the gene encoding PPAR- γ , which in turn promotes the principal steps of fat cell maturation [76–79]. However, to date, there has been no similar observation in an *in vivo* experiment. Even mice engineered for adipose tissue-restricted overexpression of a constitutively active form of PPAR- δ did not exhibit any sign of increased adipogenesis [16]. Thus, although a plausible hypothesis, empiric data do not favour PPAR- γ -like effects on adipogenesis as a cause for the PPAR- δ -induced increase in insulin sensitivity.

PPAR- δ activation increases lipid oxidation by adipocytes, which contributes to whole-body lipid dissipation in rodents, but is unlikely to do so in humans. PPAR- δ does not appear to be a key regulator of adipogenesis *in vivo*.

Antiatherogenic action

Increased cellular cholesterol efflux The effects of PPAR- δ agonists on diabetes-associated alterations in lipid handling and atherosclerosis go beyond indirect benefits that could be attributed to insulin sensitisation. In this regard, a large amount of information stems from reports of direct effects of small molecule agonists on macrophages. In these cells, PPAR- δ can function as a VLDL sensor that regulates the export and catabolism of lipids [80, 81]. Initial data suggesting that PPAR- δ agonists could have direct effects on cellular cholesterol handling came from experiments where GW501516 stimulated cholesterol efflux from cultured macrophages, fibroblasts and intestinal cells [10]. This effect was attributed at least in part to increased expression of the gene encoding the ATP-binding cassette transporter ABCA1 [10], which has been replicated in several other studies [18, 29, 82]. ABCA1 plays a critical role in the cellular transfer of cholesterol to HDL particles, which is consistent with descriptions of PPAR- δ -dependent increases in cellular cholesterol export, circulating HDL-cholesterol and ApoAI [10, 17]. A single contradictory report of lipid accumulation in macrophages may have been due to the modest subtype selectivity of the agonist employed, resulting in coactivation of the PPAR- γ lipid storage pathway [82]. In sum, the weight of evidence suggests that PPAR- δ activation should lead to protection from atherosclerosis [23, 83]. In support of this conclusion, antiatherogenic action has been documented in atherosclerosis-prone mice lacking the LDL receptor (*Ldlr*^{-/-}) that were treated with the PPAR- δ agonist GW0742 [84].

Suppression of inflammatory genes In addition to observations on cellular cholesterol handling, there are consistent results demonstrating that PPAR- δ directly downregulates

Fig. 2 PPAR- δ : a target for antiatherogenic treatment? Responses and mechanisms reported in experimental settings, which fuel speculation that PPAR- δ activation could counteract atherosclerosis and, hence, protect from congestive heart disease (CHD). Not all the indicated responses are undisputed and the antiatherogenic action of PPAR- δ is not yet established in a clinical setting. *ABCA1* ATP-binding cassette,

subfamily A, member1; *m-CPT1* muscle carnitine palmitoyltransferase-1 (also known as *CHPT1*); *LCAD* long-chain acyl-CoA dehydrogenase (also known as *ACADL*); *MCP1* monocyte chemoattractant protein 1 (also known as *CCL2*); *NPC1L1* Niemann–Pick C1-like 1; *TNF- α* (also known as *TNFAIP1*)

inflammatory genes in macrophages and atherosclerotic lesions. Elegant in vitro experiments indicated that PPAR- δ -dependent suppression of inflammatory genes in macrophages could be executed by the transcriptional repressor B cell lymphoma-6, which is released from PPAR- δ upon ligand binding [85]. In vivo, inflammatory gene repression along with inhibition of foam cell formation has been demonstrated in lesioned arteria of *Ldlr*^{-/-} mice, and this was accompanied by reduced circulating markers of inflammation. Remarkably, this effect was observed in the absence of changes in plasma HDL and persisted under experimental conditions insufficient to cause detectable changes in lesion surface [84, 86]. Although a multitude of mechanisms has been proposed that could contribute to an antiatherogenic action of PPAR- δ agonists (Fig. 2), final conclusions about their utility in the prevention or treatment of human atherogenesis cannot yet be drawn, since all the current information stems from artificial approaches using isolated cells and genetically manipulated animals.

Activation of PPAR- δ stimulates cellular cholesterol efflux and suppresses inflammatory gene expression in macrophages and atherosclerotic lesions.

Combined activation with other subtypes

Although each PPAR subtype has a specific biological function, as reflected by the pharmacological profiles of agonist action, their potential utilities as drug targets are often magnified by the clustering of diseases within an individual patient. Hence, simultaneous activation of

Fig. 3 Benefit of simultaneous activation of different PPAR subtypes. The metabolic disturbances counteracted by agonists of different PPAR subtypes often cluster in the same patient. Simultaneous activation of two or three subtypes is a rational therapeutic option. ^aPotential therapeutic effects of PPAR- δ agonists (not established)

different PPAR subtypes is a rational therapeutic option (Fig. 3). Activation of multiple PPAR subtypes could be achieved by either co-dosing of subtype-selective ligands or the development of single compounds acting as dual or triple (also called pan) agonists. The targeted design of dual agonists for PPAR- α and PPAR- γ , the glitazars, has been pursued for several years, and some of these drugs have entered the final stages of clinical development. Compounds that activate PPAR- δ together with one or both other subtypes are at an earlier stage of development. The key issue in the development of all dual and pan PPAR agonists is whether they show an improved therapeutic index compared with the subtype-selective agonists. Specifically, fluid retention seen with glitazones and glitazars, which is associated with an increase in congestive heart failure, has limited the clinical utility of these drugs. Notably, some older PPAR agonists, which were developed prior to the cloning of the receptors, may be less subtype-specific than originally assumed. For example, bezafibrate has been claimed retrospectively to exert its effects in part via PPAR- δ and to be the first, albeit weak, pan agonist in clinical use [87–89].

Dual and pan agonists of PPAR are in development. However, it is not yet known if they will have an improved ratio of efficacy : safety compared with subtype-selective agonists.

Conclusions

The availability of potent and selective PPAR- δ agonists has given rise to multiple lines of evidence that these compounds will counteract problems associated with type 2 diabetes, including insulin resistance, abnormal plasma lipid profile and increased incidence of atherosclerosis. While these insights have stimulated drug development, the full clinical potential of PPAR- δ as a drug target is far from being elucidated. Aside from the possibility that dose-limiting adverse effects could impair the utility of the drugs, our enthusiasm should be tempered by the fact that most of the projections of therapeutic utility are extrapolations from isolated cells or experimental animals, many of which were genetically engineered. While the strengths and liabilities of PPAR- δ as a drug target will soon become evident in clinical studies, the orphanisation of this receptor has already served to improve our knowledge of the physiology of lipid metabolism and whole-body fuel homeostasis.

Duality of interest T. M. Willson is an employee of GlaxoSmithKline, which has a PPAR- δ agonist under clinical development.

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