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Cardiometabolic interventions – focus on transcriptional regulators

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Abstract

Cardiovascular disease (CVD) remains the largest healthcare burden in the Western world; and the increasing prevalence of type II diabetes mellitus, at least partially driven by a trend in lifestyle changes associated with global economic development, is likely to fuel this CVD burden worldwide. Over the past two decades, there has been an increased awareness of the convergence of risk factors contributing to both cardiovascular disease and diabetes leading to the concept of the metabolic syndrome, and the realisation of the opportunity to intervene at this intersection to simultaneously target CVD and metabolic dysfunction. This brings together the fields of cardiovascular medicine, diabetology, and increasingly clinical immunology for a unified and concerted effort to reduce risk for both conditions simultaneously. The discovery of the targeted pathways of drugs already in clinical use such as fibrates and thiazolidinediones (TZD) has led to accelerated basic and clinical research into selective and dual PPAR- and PPAR- agonists, which can theoretically target glucose, lipid and lipoprotein metabolism, as well as potentially exerting inhibitory effects in vascular inflammation, all of which might be predicted to reduce atherosclerosis. In this article, we will discuss the basic science as well as recent clinical development in the pursuit of optimal cardiometabolic intervention along with insight into strategies for future drug development.

Keywords

PPAR; lipid; diabetes mellitus; obesity; metabolic syndrome; atherosclerosis; Inflammation

INTRODUCTION

Cardiovascular disease (CVD) is the most serious public health burden globally. In the Western world CVD causes 4.3 million deaths each year, representing nearly half (48%) of all-cause mortality in Europe.¹ A similar trend is also observed in the rapidly-developing countries, such as in China, where the proportion of death caused by CVD in its vast 1.3 billion population compared with all other causes of mortality was estimated to have increased from 12.8% in 1951 to 35.8% in 1990.² With the evolution in global socioeconomic activities and associated change in lifestyle, we are faced with an impending epidemic of obesity, insulin resistance, metabolic syndrome, and type II diabetes mellitus (T2DM).³ This spectrum of metabolic abnormalities confers additional risk for the

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development of microvascular and macrovascular atherothrombotic complications, which often lead to coronary heart disease and stroke.

The constellation of risk factors that constitute metabolic syndrome are well recognised by World Health Organisation (WHO), The American Heart Association/National Heart, Lung, Blood Institute (AHA/NHLBI) and the International Diabetes Foundation (IDF),⁴ and are defined in the consensus statement as visceral/abdominal obesity (enlarged waist circumference), atherogenic dyslipidaemia (elevated plasma triglyceride, reduced plasma HDL-c), elevated blood pressure, and insulin-resistant glucose metabolism (hyperinsulinemia, impaired fasting glucose, impaired glucose tolerance, T2DM).⁵ Patients with metabolic syndrome have a 5-fold increased risk of developing T2DM; even in those without diabetes, the presence of metabolic syndrome confers a 1.5 to 3-fold increased risk to develop atherosclerotic cardiovascular complications.⁶ This figure increases even further with the onset of T2DM. Therefore, as the common precursor to both CVD and T2DM, identification and treatment of metabolic syndrome have attracted the attention of both cardiologists and diabetologists.

Central to the development of metabolic syndrome is the role of insulin resistance⁷ and its relation to the progression to T2DM. Adipose tissue is the body's largest store of triglycerides; and lipolysis of adipose tissue triglycerides is the major source of plasma free fatty acids (FFA).⁸ Insulin inhibits lipolysis and serves as the major physiological regulator of adipose tissue basal lipolytic activity.^{9, 10} Although the precise mechanism remains unknown, it has been hypothesised that resistance to insulin in adipose tissue may allow unopposed lipolysis and release of FFA into the plasma.¹¹ A rise in plasma FFA not only serves as an increased flux of substrate for hepatic triglyceride and VLDL production, contributing to the atherogenic dyslipidaemia, but may also serve to stimulate fatty-acid induced insulin resistance in skeletal muscle, mediated by alteration of intracellular insulin signaling and impaired insulin-mediated glucose uptake.¹² The intricate link of insulin resistance with both atherogenic dyslipidaemia and T2DM was highlighted when the insulin-sensitisers thiazolidinediones (TZD), were found to act on the nuclear receptor peroxisome proliferator-activated receptor (PPAR) – α isoform, which has emerged to have a regulatory role in glucose, lipid and lipoprotein metabolism, as well as exerting potent anti-inflammatory effects.¹³

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR BIOLOGY

PPAR is a member of the ligand-activated nuclear receptor super-family; other members in the family include the thyroid hormone receptor and oestrogen receptor. There are three isoforms of PPARs, namely PPAR- α , PPAR- β (or δ), and PPAR- γ , each has different tissue distribution and distinct activating ligands but they share broadly similar structural domains.¹³ Upon ligand activation, PPARs form obligate heterodimers with retinoid X receptor (RXR) and act as transcription factors by binding to PPAR response element DNA in the promoter region of the target genes. While PPAR- γ is ubiquitously expressed, PPAR- α is expressed in heart and skeletal muscles, liver, and the renal cortex; whereas PPAR- β is mainly expressed in white and brown adipose tissue, cells of monocyte/macrophage lineage, the mucosa in colon and caecum, the placenta, yet almost absent in skeletal muscle.¹⁴

One key feature of PPAR signaling is the diverse downstream cellular responses observed, which is tissue/organ and agonist specific. Ligand-induced transcriptional PPAR response depends heavily on the recruitment or release of small accessory molecules (co-activators e.g. cAMP response element binding protein and p300; or corepressors e.g. nuclear co-repressor and SMRT).¹⁵ Different synthetic PPAR agonists induce a specific conformational change in PPARs with differential recruitment of co-factors, which can therefore result in a

different cellular response depending on tissue/organ context. In addition, all these multiple levels of control as seen in PPAR also exist in a similar fashion for RXR.³ Because PPARs are activated by external signals and can activate or repress entire gene cassettes in multiple pathways, Brown and Plutsky proposed that PPAR activation fits the classic definitions of a biological network nodal point, through which, in response to a specific stimulus, it might mediate a coordinated, programmed response at multiple (i.e. cellular, tissue, organ, and even organism) levels.¹⁵

PPAR AGONISTS IN CLINICAL PRACTICE

The fibrates

Fibric acid derivatives (fibrates) are PPAR- agonists. PPAR- transcription targets include genes encoding multiple proteins essential in fatty acid uptake and metabolism in metabolically active tissue such as skeletal muscle and the liver. PPAR- activation induces expression of lipoprotein lipase (LPL), which hydrolyzes triglyceride-rich circulating lipoproteins. It also represses apolipoprotein (Apo) CIII expression, which acts as the endogenous inhibitor of LPL activity.^{16,17} PPAR- activation upregulates transcription of the major HDL apolipoproteins, ApoAI and ApoAII.^{18,19} As such, fibrates demonstrate the expected biological effect in effectively reducing triglyceride levels, and raising plasma HDL-c. A number of clinical trials have evaluated fibrates in patients with or without diabetes. Gemfibrozil was shown to reduce the risk of fatal and non-fatal myocardial infarction (MI) as primary²⁰ and secondary prevention therapy,²¹ in a manner which was inversely proportional to the achieved plasma HDL-c. The effect of bezafibrate in the reduction in MI is more marked in patients with high plasma triglyceride and metabolic syndrome.^{22,23} However, recent studies of fenofibrate in patients with T2DM revealed no overall benefit in the reduction of cardiovascular mortality either used alone²⁴ or in combination with simvastatin,²⁵ although a similar trend of potential benefit is also observed in the subgroup with high plasma triglyceride and low HDL-c, suggesting a possible role in patients with metabolic syndrome.

Thiazolidinediones

Thiazolidinediones (TZDs) have been used to restore insulin sensitivity in the treatment of T2DM since the late 1990s.²⁶ This class of agents are activators of PPAR- and appear to be beneficial in surrogate marker studies of cardiovascular disease.^{27,28} PPAR- is essential in adipogenesis and is a key regulator to control lipid metabolism.²⁹ It regulates genes for lipoprotein lipase, acyl-coenzyme A synthetase, adipocyte-restricted intracellular lipid-binding protein aP2, and for glucose control such as the glucose transporter GLUT4 and phosphoenolpyruvate carboxykinase.³⁰ Activation of PPAR- in adipose tissue is thought to mediate its insulin sensitising effects. Although the precise mechanisms remain undefined, induction of fat cell redistribution from large insulin-resistant adipocytes to smaller, more insulin-sensitive cells appeared important.³¹ PPAR- activation also leads to reduced release of FFA and insulin-resistance-mediating adipokines, such as tumor necrosis factor (TNF)- α , leptin and resistin; while increasing production of anti-inflammatory and anti-diabetic adipokine adiponectin, which may reduce progression of metabolic syndrome.³²

Importantly, PPAR- is also expressed in human monocytes/macrophages.³³ Macrophages play a key regulatory role in the initiation, progression, as well as experimental regression of atherosclerosis.³⁴ PPAR- has long been shown as a negative regulator of macrophage activation by antagonising the activities of transcription factors AP-1, STAT, and NF- κ B.³⁵ Recent evidence has shown that in addition to its effect in lipid uptake via up-regulation of CD36, PPAR- activation in human macrophages also activates the cellular machinery of cholesterol efflux. It increases the expression of the key lipid transporters ATP-binding

cassette transporters ABCA1 and ABCG1 through a transcription cascade mediated by another nuclear receptor liver X receptor (LXR)-³³ and promotes functional reverse cholesterol transport (RCT) from macrophage-derived foam cells.³⁶ This is thought to contribute to the overall atheroprotective effect of PPAR- since ABC transporters-mediated RCT is important in experimental regression model of atherosclerosis³⁷ and that the ABCA1 is directly implicated in regulating macrophage responsiveness to toll-like receptor (TLR) agonists by modulation of lipid raft cholesterol and MyD88-dependent TLR mobilisation to lipid rafts.³⁸

The theoretical cardiovascular benefit of TZDs has led to a number of clinical studies in patients with T2DM but with contradictory outcomes. In the ProACTIVE trial,³⁹ pioglitazone was shown to significantly reduce its main secondary composite endpoint of all-cause mortality, non-fatal MI, and stroke (16% relative risk reduction, $P=0.027$) amongst T2DM patients with previously established macrovascular disease. Although this statistical significance was narrowly lost when coronary and peripheral revascularisation was included in its primary composite endpoints (10% relative risk reduction, but $P=0.095$), subsequent post-hoc analysis revealed that all 'disease-related' endpoints (mortality, MI, stroke, ACS, leg amputation) decreased significantly ($P=0.0087$), whereas 'procedure-related' endpoints increased (leg revascularisation) or were similar (coronary revascularisation) in the pioglitazone versus placebo groups.⁴⁰ The benefit of pioglitazone in the secondary prevention of stroke and MI in non-diabetic patients, however, remains to be established in an on-going trial.⁴¹

However, the use of TZDs as a cardiometabolic intervention was seriously challenged when subsequent data suggested worrying safety profile.⁴² In addition to hepatotoxicity caused by troglitazone,⁴³ bone fracture and heart failure are associated with the use of rosiglitazone,^{44,45} and similar findings were confirmed for pioglitazone by analysis of safety reports by the manufacturer Takeda.⁴⁶ Pharmacovigilance programme has also identified an association between pioglitazone and bladder cancer,⁴⁷ a finding supported by a recent nested case control study.⁴⁸ Even at the time of licencing, fluid retention and risk of congestive heart failure with the use of TZDs was already recognised.⁴⁹

Concern over cardiovascular safety of rosiglitazone was further highlighted by a meta-analysis, which showed significant increase in myocardial infarction and increased risk of cardiovascular death.⁵⁰ To address concern, the RECORD trial was set up specifically to evaluate the cardiovascular outcome and safety of rosiglitazone.⁵¹

While it confirmed the doubling of risk of heart failure with rosiglitazone, it failed to draw a conclusive closure on CV safety due to issues with study design and event adjudication.⁵² When the US Food and Drug Administration (FDA) explored a large (227,571) cohort of the Medicare database, rosiglitazone was found to be associated with increased risk of stroke, heart failure, and death and the composite of non-fatal MI, stroke, heart failure, or death.⁵³ Consequently, the European Medicines Agency (EMA) has withdrawn rosiglitazone from the European market⁵⁴ and its use was severely restricted by the FDA.

Dual PPAR- α/γ agonist – the emerging candidates?

Since fibrates (PPAR- α agonists) exert favourable effects on plasma lipoproteins, and TZDs (PPAR- γ agonists) improve insulin sensitivity and inflammatory biomarkers – all of which represent key components in metabolic syndrome – there has been an increasing interest in exploring the clinical effects of combined dual PPAR- α/γ agonism. A key feature of PPAR signaling is that distinct PPAR agonists can alter the PPAR conformation in a specific way to differentially recruit co-factors that activate or repress specific transcription cascade.¹⁵ Therefore each PPAR agonist has its unique "compound effect" as well as a "class effect".

Development of several dual PPAR- / agonists was terminated in late-phase trials due to specific safety concerns with increased cardiovascular risk (muraglitazar), renal impairment (tesaglitazar), and carcinogenicity (ragaglitazar and KRP-297/MK-076).⁵⁵ Some researchers suggested that the balanced binding affinity of PPAR- / dual agonists towards both the receptor subtypes is necessary to give optimal biological effects of both PPAR- -mediated and PPAR- -mediated actions;⁵⁶ and that supra-therapeutic activation of PPAR- (e.g. tesaglitazar) or PPAR- (e.g. muraglitazar) may be associated with adverse effects.

This led to the recent development of a balanced PPAR- / agonist aleglitazar.⁵⁷ After encouraging preclinical and phase I study, the effect of balanced stimulation of PPAR- and PPAR- by aleglitazar was evaluated in SYNCHRONY,⁵⁸ a 16-week phase II clinical trial in patients with T2DM designed to evaluate optimal dosing and safety profile. After a wash-in period, patients were randomised to receive varying doses (50, 150, 300, or 600 µg) of aleglitazar versus placebo or open-labelled pioglitazone at standard dose (45mg). Interestingly, aleglitazar, even at lower dose of 150 µg, exert similar or greater effect on HbA1c, plasma fasting glucose, as well as plasma lipoprotein (triglycerides, HDL-c, LDL-c, and apolipoprotein B) compared with pioglitazone. Adverse effect profile of aleglitazar, in the small sample size (n = 332) of SYNCHRONY, appeared comparable to that of pioglitazone, with peripheral oedema being the most common. However, adjudicated heart failure was only reported in two patients on high (300 and 600 µg) doses. Importantly, there was no myocardial infarction or stroke recorded in the study.

Although SYNCHRONY was too small a study to evaluate the definitive safety profile of aleglitazar, there is no early signal to suggest an increase in cardiovascular events, which was noted with patients after short exposure to rosiglitazone 50 and mugaglitazar.⁵⁹ Additionally, the renal safety of aleglitazar appeared to be encouraging.

Although there is a dose-dependent fall of estimated glomerular filtration rate (eGFR) across the dose range in SYNCHRONY (4% to 14%), two subsequent studies have shown that the renal effects of aleglitazar are stable and reversible in T2DM patients whose renal function is mildly⁶⁰ or moderately⁶¹ impaired. A number of phase III trials of aleglitazar are now in progress. AleCardio is an outcome trial evaluating the effect of balanced PPAR- / action of aleglitazar on secondary prevention in T2DM patients after acute coronary syndrome; it is expected to report in 2015.⁶² AlePREVENT is another outcome trial to evaluate aleglitazar as a cardiovascular primary prevention therapy in T2DM patients with stable cardiovascular disease.⁶³ Finally, AleGlucose is a set of glycaemic control trials to further characterise the impact of aleglitazar on glycaemic control in patients with T2DM. Taken together, emerging data has suggested that a balanced dual PPAR- / agonism may improve glycaemic control and reverse atherogenic dyslipidaemia, with a relatively favourable adverse-effect profile. Whether this new class of dual PPAR- / drug can deliver the theoretical promise seen in pre-clinical studies in reducing cardiovascular risk in patients with metabolic syndrome or T2DM will largely depend on careful outcome trials that will emerge in the next few years.

OTHER CARDIOMETABOLIC INTERVENTION

Endocannabinoids system

The endocannabinoid system is comprised of two G-protein coupled receptor (GPCR) CB1 and CB2, named after the major psychotropic component of Cannabis sativa, 9-tetrahydrocannabinol (THC), which activates them. The endogenous cannabinoid ligands, known as endocannabinoids, include N-arachidonoyl-ethanol-amine (anandamide) and 2-arachidonoyl-glycerol (2-AG). Dysregulation of the endocannabinoid system has been implicated in the loss of energy homeostasis, body weight control, as well as adipose tissue distribution, contributing to abdominal (central) obesity and subsequent resistance to insulin

and metabolic syndrome.^{64,65} More recently, it has also been shown to exert a key regulatory role in endothelial function,^{66,67} cardioprotection in ischaemic-reperfusion injury,⁶⁸ as well as in atherosclerosis and inflammation.^{69,70} The likely pathophysiological consequence of unopposed endo-cannabinoid or CB1 tone is an increase in the levels of inflammatory cytokines, with macrophage activation, coupled with disequilibrium in energy homeostasis and subsequent pro-atherogenic dyslipidaemia.⁷¹ There has been recent attempt to develop a CB1 inverse agonist/antagonist rimonabant, which had shown promising results in improving body weight and metabolic and inflammatory abnormalities in several phase III trials in obese subjects.⁷²⁻⁷⁴ However, the increased incidence of adverse psychiatric complications, likely related to CB1 effect in the brain, has prompted the withdrawal of rimonabant from the market by the European Medicines Agency.⁷⁵

Incretin-based therapy

Incretins are a group of gut hormones secreted in response to enteral nutrient ingestion, which augment the insulin response by pancreatic β -cells.^{76,77} The two most studied incretins are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). The bioavailability of GLP-1 (and GIP) is limited by the rapid action of the enzyme dipeptidyl peptidase 4 (DPP-4), which inactivates native GLP-1 within minutes after its secretion.⁷⁸ This has led to the strategic development of GLP-1 analogues, which are insensitive to DPP-4 inactivation, as well as direct DPP-4 inhibitors, to restore the blunted/attenuated incretin response in patients with T2DM. In addition to satiety, body weight, and glucose metabolic control, there is emerging evidence to suggest a direct cardiovascular benefit from incretin-based therapy.⁷⁹ GLP-1 receptors are expressed in the endothelium, as well as cardiomyocytes and vascular smooth cells.⁸⁰

Cell culture and animal studies have shown that activation of endothelial GLP-1 receptors inhibit the TNF- α mediated expression of plasminogen activator inhibitor-1, which is characteristically elevated early on in diabetic patients with endothelial dysfunction;⁸¹ GLP-1 analogue liraglutide treatment in mice increased endothelial nitric oxide synthase (eNOS) and reduced intercellular adhesion molecule-1 (ICAM-1) expression in aortic endothelium.⁸² Similar positive cardiovascular effects was seen with DPP-4 inhibitor alogliptin in ApoE $-/-$ mice which reduced TLR-4 mediated expression of IL-6 and IL-1 in atherosclerotic plaque, and reduced pro-inflammatory cytokines release by mononuclear cells, as well as diminished overall plaque burden in diabetic mice.⁸³ In the myocardium, liraglutide was shown to have an anti-apoptotic effect by reducing caspase-3 cleavage.⁸⁴ Early human studies have shown that GLP-1 receptor activation resulted in larger myocardial salvage after primary percutaneous intervention following acute myocardial infarctions,⁸⁵ and may possibly improved global and regional left ventricular function post-infarct.⁸⁶ At present, the majority of GLP-1 receptor analogues and DPP-4 inhibitors are being assessed in large multi-centre clinical trials for cardiovascular outcomes;⁸⁷ with some studies of DPP-4 inhibitors either completed recruitment (vildagliptin),⁸⁸ or expected to complete in 2013 (SAVOR-TIMI 53⁸⁹; EXAMINE⁹⁰).

CONCLUSION

The increasing awareness of the convergence of risk factors contributing to both cardiovascular disease and metabolic derangement has led to the recognition and rapid expansion of cardiometabolic intervention. The recent discovery of various potentially immunomodulatory G-protein coupled receptors signaling cascades with endogenous "metabolic" ligands such as fatty acid, saccharides, lactate and ketone bodies, has further highlighted the degree of "cross-talk" between metabolic and inflammatory pathways, both intricately linked with cardiovascular diseases.⁹¹ There is strong theoretical rationale and pre-clinical evidence for the pharmaceutical pursuit of newer multi-action drugs such as dual

PPAR- / agonists and incretin-based therapy; but pre-clinical benefits may not always translate to the clinic and adverse effects may be unpredictable. As such, we eagerly await a number of large cardiovascular outcome trials to evaluate their true translational potential. By bringing together the fields of cardiovascular medicine and diabetology, we should see a more unified and concerted effort to combat cardiometabolic risks in the future.

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