Review

Pioglitazone and mechanisms of CV protection

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Summary

Background and Aim: Pioglitazone has diverse multiple effects on metabolic and inflammatory processes that have the potential to influence cardiovascular disease pathophysiology at various points in the disease process, including atherogenesis, plaque inflammation, plaque rupture, haemostatic disturbances and microangiopathy.

Results: Linking the many direct and indirect effects on the vasculature to the reduction in key macrovascular outcomes reported with pioglitazone in patients with type 2 diabetes presents a considerable challenge. However, recent large-scale clinical cardiovascular imaging studies are beginning to provide some mechanistic insights, including a potentially important role for improvements in high-density lipoprotein cholesterol with pioglitazone. In addition to a role in prevention, animal studies also suggest that pioglitazone may minimize damage and improve recovery during and after ischaemic cardio- and cerebrovascular events.

Design and Methods: In this review, we consider potential cardiovascular protective mechanisms of pioglitazone by linking preclinical data and clinical cardiovascular outcomes guided by insights from recent imaging studies.

Conclusion: Pioglitazone may influence cardiovascular disease pathophysiology at multiple points in the disease process, including atherogenesis, plaque inflammation, plaque rupture and haemostatic disturbances (i.e. thrombus/embolism formation), as well as microangiopathy.

Introduction

For many years it was hypothesized that thiazolidinediones (TZDs) might improve clinical cardiovascular (CV) outcomes based on their insulin-sensitizing mechanism of action, favourable metabolic profiles, impact on multiple CV biomarkers and observations in animal studies.¹⁻⁴ This concept has now been formally tested in large-scale CV outcomes and imaging studies with pioglitazone. The results of PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) provide evidence that pioglitazone therapy is associated with a reduced risk of macrovascular events in patients with type 2 diabetes and pre-existing macrovascular disease.⁵⁻⁸ Atherosclerotic disease (with subsequent plaque rupture and thrombus/embolus formation) represents the key underlying pathophysiology for the majority of ischaemic macrovascular events, and imaging studies have demonstrated an anti-atherogenic effect of pioglitazone that is likely to explain, at least in part, the findings from PROactive.⁹,¹⁰ Preclinical data also suggest that

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pioglitazone may have other CV-protective properties beyond the prevention of macrovascular events, such as minimizing ischaemic damage to the myocardium and neuronal tissue after occlusive vascular events.

Pioglitazone affects a diverse array of metabolic and inflammatory processes potentially relevant to CV disease pathophysiology, including key metabolic risk factors (dyslipidaemia, hyperglycaemia, hypertension), insulin resistance, endothelial dysfunction and inflammatory cytokines, markers of plaque stability, adhesion molecules and mediators of coagulation/fibrinolysis. However, it remains unclear which part of this complex profile is most relevant to the potential macrovascular benefits and anti-atherosclerotic effects reported in PROactive.

In this review, we consider potential CV protective mechanisms of pioglitazone by linking preclinical data and clinical CV outcomes guided by insights from recent imaging studies. In light of the recent controversy over potential differences between individual TZDs in terms of their effects on CV outcomes and the divergent effects of individual agents on lipid profiles and gene expression, we will focus specifically on pioglitazone-associated effects rather than TZD- or peroxisome proliferator-activated receptor-gamma (PPARγ)-associated effects.

Pioglitazone and macrovascular outcomes—PROactive, CHICAGO and PERISCOPE

With the completion of the PROactive, Carotid intima-media thickKness in Atherosclerosis using pioglitazOne (CHICAGO) and Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) studies, pioglitazone probably represents the best studied of all oral glucose-lowering therapies in terms of CV outcomes. The striking congruence of the results from these three studies provides a sound basis for considering the potential macrovascular benefits of pioglitazone.

PROactive was a prospective, multicentre, randomized, double-blind, placebo-controlled outcome study that investigated the effects of pioglitazone on macrovascular outcomes in >5000 high-risk patients with type 2 diabetes and pre-existing macrovascular disease treated with guideline-driven therapy for multiple risk factors. Pioglitazone was administered at a dose of 15 mg/day for the first month, 30 mg for the second month, and 45 mg thereafter to achieve the maximum tolerated dose, according to the licensed dose range for pioglitazone (89% of patients reached the 45 mg dose at 2 months, and at least 93% of those continuing on pioglitazone received the highest dose). Over ~3 years (a relatively short duration for a CV outcomes trial), pioglitazone treatment was associated with a statistical trend towards benefit compared with placebo [19.7% vs. 21.7%; hazards ratio (HR) = 0.90, 95% confidence interval (CI) 0.80–1.02, P = 0.095] for the primary composite macrovascular endpoint (which included cerebral, cardiac and peripheral events and both disease-related and procedural endpoints). Importantly, however, a statistically significant reduction in the main secondary endpoint of the key composite of all-cause mortality, myocardial infarction (MI) and stroke (11.6% vs. 13.6%; HR = 0.84, 95% CI 0.72–0.98, P = 0.027) was also reported, along with significant effects on a range of other major adverse cardiovascular event (MACE) composite endpoints, most notably the frequently-used pre-specified composite of CV mortality, MI and stroke (9.9% vs. 11.9%; HR = 0.82, 95% CI 0.70–0.97, P = 0.020). Of note, there appeared to be particularly robust significant reductions in the risk of recurrent MI (5.3% vs. 7.2%; HR = 0.72, 95% CI 0.52–0.99, P = 0.0453) and recurrent stroke (5.6% vs. 10.2%; HR = 0.53, 95% CI 0.34–0.83, P = 0.009). These results are consistent with a recent meta-analysis of CV adverse events from pioglitazone clinical efficacy/safety trials, irrespective of whether PROactive was included in the analysis.

The two subsequent large-scale imaging studies (CHICAGO and PERISCOPE) provided results consistent with the suggestion of a macrovascular benefit of pioglitazone seen in PROactive. In the CHICAGO study, which involved 462 patients with type 2 diabetes (most of whom had no history of cardiovascular disease (CVD)), pioglitazone 15–45 mg/day [titrated to achieve fasting plasma glucose ≤140 mg/dl (7.8 mmol/l)] significantly slowed the progression of carotid intima-media thickness (CIMT), a validated measure of atherosclerosis progression, compared with glimepiride 1–4 mg/day over 18 months (Figure 1). This result confirmed earlier observations of significant reductions in CIMT progression with pioglitazone vs. comparators in smaller less rigorous imaging studies and a recent analysis of imaging data from the Pioglitazone in Prevention of Diabetes (PIPOD) study (a single-arm, 3-year, open-label study of pioglitazone treatment in women with prior gestational diabetes who had completed the Troglitazone in Prevention of Diabetes—TRIPOD study). In the original TRIPOD study, CIMT progressed significantly in the placebo arm, but increased only...
Figure 1. Recent imaging results suggest significant vascular benefits with pioglitazone.⑨⑩⑱ (A) Pioglitazone slows progression of mean CIMT in patients with type 2 diabetes—primary outcome from the CHICAGO study (n = 462).⑨ Reproduced with permission from Mazzone et al. JAMA 2006;296:2572–2581. Copyright © 2006 American Medical Association. All rights reserved. (B) Pioglitazone slows progression of coronary atherosclerosis (measured over as change percent atheroma volume over 18 months using IVUS in patients with type 2 diabetes and coronary disease—primary outcome from PERISCOPE (n = 543).⑩ Figure derived from data. (C) Pioglitazone rapidly improves carotid plaque echolucency (a measure of plaque vulnerability) assessed using carotid ultrasound with integrated backscatter in patients with acute coronary syndrome and type 2 diabetes (n = 61).⑱ *p < 0.01 vs respective baseline values, †p < 0.01 vs values in patients with stable coronary artery disease. Reprinted from Atherosclerosis, Vol. 203, Hirano M, Nakamura T, Kitta Y, Yano T, Kobayashi T, Sano K, et al., Rapid improvement of carotid plaque echogenicity within 1 month of pioglitazone treatment in patients with acute coronary syndrome, 483–8, Copyright (2009), with permission from Elsevier. CIMT = carotid intima-media thickness; IBS = integrated backscatter; IVUS = intravascular ultrasound; PAV = percent atheroma volume.
slightly (and non-significantly) in the troglitazone arm. In the subjects who came to PIPOD from the placebo arm of TRIPOD, the rate of CIMT was significantly lower with pioglitazone than it had been during placebo, whereas in those who came from the troglitazone arm, the relatively low rate of progression seen during TRIPOD was at least maintained with pioglitazone. The recent PERISCOPE study used the intravascular ultrasound (IVUS) technique to assess directly the progression of coronary atherosclerosis in 543 patients with type 2 diabetes and coronary disease. As in CHICAGO, pioglitazone 15–45 mg/day significantly slowed the progression of atherosclerosis (quantified by the change in percent atheroma volume) compared with glimepiride 1–4 mg/day over 18 months (Figure 1).10

Another interesting recent placebo-controlled imaging study has used ultrasound to examine the effects of pioglitazone on the echolucency of carotid arterial plaques (as an index of plaque vulnerability) in 61 Japanese patients with type 2 diabetes and acute coronary syndrome. Pioglitazone rapidly and significantly improved carotid plaque echolucency relative to baseline within 1 month of therapy initiation, suggesting that it might have an early benefit in stabilizing unstable carotid plaques, whereas no significant improvements were seen with placebo (Figure 1). These effects correlated with changes in inflammatory markers (CRP, TNFα), insulin sensitivity and adiponectin, and are consistent with the beneficial effects seen on markers of plaque instability (see below).

Studies showing a significant reduction in rates of restenosis and target vessel revascularization in percutaneous coronary intervention patients treated with pioglitazone provide further clinical evidence for a vascular benefit. Thus, the results of PROactive alone are at least suggestive of a macrovascular benefit with pioglitazone, and this is strengthened further when these results are considered alongside the anti-atherosclerotic effects of pioglitazone reported in CHICAGO, PERISCOPE and other smaller studies.

Potential mechanisms underlying the cardiovascular benefits of pioglitazone

**Improved insulin sensitivity and reduced hyperinsulinaemia**

Insulin insensitivity is known to be associated with accelerated atherosclerosis and appears to be a risk marker for both MI and stroke. The impact of insulin resistance on the vasculature may involve systemic metabolic, pro-inflammatory disturbances due to dysfunctional insulin signalling in other tissues (e.g. adipose tissue, liver), or it may be a more direct consequence of dysfunctional insulin signalling in the vasculature itself.

In insulin-resistant states, it appears to be primarily the metabolic, anti-inflammatory, antiatherogenic actions of insulin (those occurring via the PI-3-kinase/Akt dependent signalling pathway) that are impaired in many tissues (including the endothelial cells, vascular smooth muscle cells (VSMCs) and macrophages in the vasculature), rather than its mitogenic, pro-inflammatory, pro-atherogenic actions (those occurring via the MAP kinase dependent pathways), which generally remain intact. This potentially injurious state may be compounded further by the compensatory hyperinsulinaemia that occurs as the body attempts to maintain glycaemic control, thus leading to overstimulation of the insulin signalling pathways that remain sensitive in type 2 diabetes. These effects may occur alongside activation of NF-κB and MAP kinase pathways due to systemic pro-inflammatory stimuli secondary to insulin resistance in other metabolic tissues.

Although different TZDs have diverse, complex, non-overlapping metabolic actions involving the up- or down-regulation of hundreds of genes, insulin sensitization in metabolic tissues and the vasculature is their common defining characteristic. Pioglitazone appears to be selective in its action on insulin signalling by improving impaired insulin activation of PI-3-kinase/Akt-dependent signalling without increasing MAP kinase-dependent signalling or mitogenic effects. In fact, there is evidence that pioglitazone can inhibit insulin activation of MAP kinase-dependent effects (e.g. MMP-9 production and monocyte migration). Pioglitazone also has a well-characterized ability to reduce hyperinsulinaemia (both fasting and post-meal insulin levels) significantly in patients with type 2 diabetes, consistent with its insulin-sensitizing action.

**Improved glycaemic control**

As an effective glucose-lowering agent, pioglitazone has the potential to reduce the risk of microvascular disease associated with hyperglycaemia. The UK Prospective Diabetes Study (UKPDS), the Kumamoto and, more recently, the Action in Diabetes and Vascular disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) studies all suggest that more intensive glucose control can reduce the risk of retinopathy, nephropathy or neuropathy in patients with type 2 diabetes.
and there appears to be a long-term legacy effect over several decades. Although the effects of pioglitazone have not been investigated specifically in a microvascular outcomes trial, there is evidence from several sources to suggest that it may have a beneficial impact, principally in terms of slowing the progression of nephropathy, which is in itself an independent risk factor for CVD. For instance, pioglitazone has been shown to reduce urinary albumin excretion in patients with type 2 diabetes with either normo- or micro-albuminuria, and to delay the progression of chronic renal failure when added to losartan in patients with diabetic nephropathy.

Although an association between glycaemia and macrovascular outcomes is evident in epidemiological studies, the potential impact of glucose lowering on macrovascular outcomes remains controversial, particularly in the light of recent outcomes studies. In the UKPDS, there was a reduction in the risk of MI (but not stroke) that just failed to reach significance with intensive vs. less intensive glucose control in newly diagnosed patients with type 2 diabetes, although this did reach significance after 10 years of post-trial follow-up (even though randomized therapy had ended and levels of glucose control had equalized between the groups). It remains unknown whether this result would have been any better or worse if randomized therapy had been continued. A significant reduction in the risk of MI seen among overweight patients treated with metformin in the UKPDS appeared to be independent of glucose control. However, this finding for metformin is based on a relatively small number of patients (n = 342; only 8% of the total UKPDS population) and has not been demonstrated in prospective, controlled outcome studies. In the Kumamoto study (which compared intensive and conventional insulin regimens over 6 years), the population size (n = 110) and number of events (n = 6) were too small to make any firm conclusions regarding effects on macrovascular risk, although there were fewer events in the intensive control group.

The controversy over glycaemic control and macrovascular outcomes has been compounded recently by the results from the ADVANCE and Action to Control Cardiovascular Risk in Diabetes (ACCORD) studies. In ADVANCE, intensive glycaemic control (using a gliclazide-based regimen) provided no macrovascular benefit vs. less intensive control, despite impressive improvements in HbA1c. A similar result was obtained with even more aggressive and more rapid glucose control in the ACCORD study—indeed, the intensive arm was halted early due to a significant increase in all-cause mortality. Although several plausible explanations underlying the worse outcomes associated with intensive glycaemic control in ACCORD have been proposed (e.g. more hypoglycaemia, greater weight gain, greater use of specific medications and increased patient stress), none are currently supported by statistical analyses. Nevertheless, both ACCORD and ADVANCE appeared to show some reduction in the risk of macrovascular events emerging after ~5 years, as seen in the separation of the Kaplan-Meier curves at this point. Along with the results of the UKPDS post-trial monitoring, these studies suggest that any benefits of glucose lowering on macrovascular outcomes may take many years to become apparent. Although PROactive was not a glucose control study (patients in the placebo group were also being treated according to similar guidelines), the inclusion of pioglitazone in the active treatment group, provided a significantly greater improvement in glycaemic control (a 0.5% reduction in HbA1c relative to placebo). However, what is remarkable about PROactive is that the benefits (e.g. for the composite of CV death, MI and stroke, similar to that used for ADVANCE and ACCORD) of pioglitazone emerged well within the 3-year duration of the study (after ~12 months).

**Improved atherogenic lipid profile—are effects on HDL-cholesterol key to the anti-atherogenic effects of pioglitazone?**

Pioglitazone significantly improves the atherogenic lipid profile that characterizes type 2 diabetes, with effects on small dense low-density and high-density lipoprotein (LDL and HDL) cholesterol and triglyceride-rich lipoprotein particles. In recent years, much has been learned about the mechanisms underlying the anti-atherogenic properties of HDL-cholesterol. These are highly complex and may involve anti-oxidant, anti-thrombotic, anti-inflammatory, anti-apoptotic and anti-infective properties, as well as effects on endothelial function and repair. In particular, HDL is involved in the promotion of cholesterol efflux (‘reverse cholesterol transport’) from the arterial wall to the liver for excretion. In atherosclerotic lesions, this relates primarily to macrophages, which are the main cells overloaded with cholesterol.

HDL comprises a heterogeneous family of particles with varying and distinct properties, and the ability of HDL to protect the vasculature may reflect the quality of HDL in terms of its subfractional composition, as well as the function of individual components. Dysfunctional HDL is evident in patients with established coronary heart disease (CHD) and may be an important factor in the
accelerated atherosclerosis seen in certain metabolic diseases, such as type 2 diabetes. The capacity for an intervention to improve the overall function of HDL may therefore be a more important determinant of the impact on CV outcomes than the absolute levels of total HDL achieved per se.2

The ability of pioglitazone to increase HDL levels by ~10–20% from baseline (~10% vs. placebo or other oral glucose-lowering agents) is now well established when it is used either as monotherapy or when added to other oral agents.44,53 Pioglitazone appears to increase HDL by stimulating the de novo hepatic synthesis of apoA-I without affecting hepatic HDL-protein or HDL cholesterol removal (an effect that may involve weak effects on PPARα).54 A recent analysis from the CHICAGO study suggests that this increase in HDL may be a particularly important factor underlying the effects of pioglitazone on atherosclerosis progression. Of all the on-treatment changes in metabolic parameters, only the increase in HDL cholesterol and decrease in insulin levels predicted the slowing of CIMT progression seen with pioglitazone.55 Similar analyses from the PERISCOPE and PROActive trials are underway and should provide further insight on this apparent association.

In addition to its effect on HDL, pioglitazone treatment is also associated with a well-established 15–20% reduction in fasting triglyceride levels, due to a significant decrease in the amount of triglyceride present in very low-density lipoprotein (VLDL), which may relate to a reduction in large VLDL particle concentration and a decrease in mean VLDL particle size.44,45,53,56 Post-prandially, pioglitazone is also associated with a significant improvement in the disposal of chylomicrons, and in atherogenic chylomicron remnant metabolism; this, coupled with the reduction in fasting VLDL and triglycerides, is responsible for a 35% improvement in post-prandial triglyceride clearance.44 Pioglitazone (used as monotherapy or combination therapy) also reduces levels of atherogenic small dense LDL3 particles, while raising levels of larger and less-atherogenic LDL fractions.57–60 Notably, the differential effects of pioglitazone on LDL subfractions are complimentary and additive to those of simvastatin.61

Elevated free fatty acids (FFA) represent another aspect of dyslipidaemia in type 2 diabetes and insulin resistance that may not receive as much attention as other more conventional lipid risk factors. Increased plasma FFAs are associated with tissue fat accumulation, leading to activation of the pro-inflammatory NFκB pathway and the expression of pro-inflammatory and pro-atherogenic cytokines, as well as decreased nitric oxide (NO) production in endothelial cells (for review, see Boden, 2008).62 They may also influence these processes directly via plasma membrane toll-like receptors. Long-term treatment with pioglitazone is associated with improved FFA sensitivity to insulin and significantly reduced fasting FFA levels.63

**Inflammatory processes and atherosclerosis**

Inflammation is considered central to the initiation and progression of atherosclerosis. This may be particularly important in type 2 diabetes where atherosclerotic plaques appear to have a significantly higher inflammatory status with a greater infiltration of macrophages, T-lymphocytes and other activated inflammatory cells.65,66 Further evidence for a link between C-reactive protein (CRP) and CVD has been provided in a recent study of patients with high baseline CRP and low LDL cholesterol—rosuvastatin provided a significant anti-atherosclerotic effect (resulting in a lower relative risk of cardiovascular events and mortality than placebo), probably by reducing inflammation as measured by a decrease in CRP levels.67 Via its effects on cells in adipose tissue and the liver, pioglitazone improves circulating levels of a range of biomarkers of inflammation and CVD, such as CRP, adiponectin, monocyte chemoattractant protein-1 (MCP-1), MMP-9 (a marker of plaque instability) and PAI-1 (Table 1).53,59,63,68–72,86,88 However, pioglitazone also appears to have a direct influence on inflammatory processes in the vasculature. A recent study in pigs has shown directly that pioglitazone can inhibit inflammatory cell infiltration to the injured vessel wall via the inhibition of an NFκB dependent MCP-1 mediated pathway.91 In particular, pioglitazone directly influences three key vascular cell types believed to play a role in atherogenesis—endothelial cells, VSMCs and macrophages/monocytes (Figure 2).68,92 Firstly, in endothelial cells, pioglitazone inhibits endothelial inflammation and monocyte adhesion and reduces endothelial dysfunction. For instance, pioglitazone reduces both basal and TNFα-induced increases in RAGE expression (making cells less susceptible to the pro-inflammatory effects of AGE) via inhibition of the key proinflammatory transcription factor NFκB.93 Pioglitazone can reduce monocyte-endothelial cell binding by inhibiting VCAM-1 expression on activated endothelial cells (and neutrophil-endothelial cell binding by inhibiting upregulation of CD11b/CD18 on activated neutrophils).94–96 Notably, the effect on VCAM-1 may occur via a PPARγ-dependent mechanism.96 Pioglitazone also reduces ox-LDL, Ang II and
In patients with type 2 diabetes, pioglitazone improves circulating levels of key systemic markers with a potential role in CVD.\(^{23,34,35,47–49,51,53,59,63,69,72,74–90}\)

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<th>Metabolic parameter</th>
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<tr>
<td>Glucose (FPG/HbA(_1c))</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, plaque rupture, thrombogenesis, ischaemic tissue injury • Robust predictor of CV risk: continuous relationship exists between hyperglycaemia and CVD • Lowering glucose reduces microvascular risk and (probably) macrovascular risk</td>
<td>Decrease</td>
<td>Chiquette et al., 2004; UKPDS 33, 1998; Stratton et al., 2000; Rydén et al., 2007; ADVANCE Collaborative Group, 2008(^{23,34,35,53,74})</td>
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<td>Hyperinsulinaemia</td>
<td>• Preferentially activates proinflammatory/proatherogenic signaling pathways in the presence of insulin resistance</td>
<td>Decrease</td>
<td>Pfutzner et al., 2005; Muniyappa et al., 2007(^{69,75})</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>• Potential protective role against atherosclerotic lesion formation, plaque rupture, thrombogenesis • Promotes cholesterol efflux; multiple other anti-inflammatory/antiatherosclerotic properties • Component of the ‘atherogenic lipid triad’ • Robust predictor of CV risk (inverse relationship)</td>
<td>Increase</td>
<td>NCEP, 2002; Chiquette et al., 2004; Pfützner et al., 2005; Barter &amp; Ryde, 2006; Kontush et al., 2006a; Kontush et al., 2006b; Rader et al., 2006; Forst et al., 2008(^{47–49,51,53,74})</td>
</tr>
<tr>
<td>sdLDL</td>
<td>• Highly atherogenic subspecies of LDL cholesterol • Component of the ‘atherogenic lipid triad’ • Robust predictor of CV risk</td>
<td>Decrease</td>
<td>NCEP, 2002; Goldberg et al., 2005; Rizzo &amp; Berneis, 2006; Rizzo et al., 2008(^{59,76–78})</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>• Reflect the presence of atherogenic remnant lipoproteins • Component of the ‘atherogenic lipid triad’ • Robust predictor of CV risk</td>
<td>Decrease</td>
<td>Chiquette et al., 2004; Pfützner et al., 2005; Forst et al., 2008; NCEP, 2002; Jacobsen et al., 2007(^{53,69,72,76,79})</td>
</tr>
<tr>
<td>CRP</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, plaque rupture, thrombogenesis, ischaemic tissue injury • Robust predictor of CV risk</td>
<td>Decrease</td>
<td>Pfützner et al., 2005; Forst et al., 2008; Bosoendial et al., 2007; Dotsenko et al., 2008; Packard &amp; Libby, 2008(^{69,72,80–82})</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>• Potential protective role against atherosclerotic lesion formation, plaque rupture, thrombogenesis, ischaemic tissue injury • Inconsistent predictor of CV risk (inverse relationship)</td>
<td>Increase</td>
<td>Pfützner et al., 2005; Hopkins et al., 2007; Betowski et al., 2008; Dotsenko et al., 2008; Forst et al., 2008; Packard &amp; Libby, 2008(^{69,72,81–84})</td>
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<td>MMP-9</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, plaque rupture, thrombogenesis • Predictor of CV risk</td>
<td>Decrease</td>
<td>Pfützner et al., 2005; Forst et al., 2008; Packard &amp; Libby, 2008(^{69,72,82})</td>
</tr>
<tr>
<td>FFA</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, ischaemic tissue injury • Predictor of CV risk</td>
<td>Decrease</td>
<td>Pfützner et al., 2005; Roden et al., 2008; Pilz &amp; März, 2008(^{83})</td>
</tr>
<tr>
<td>PAI-1</td>
<td>• Potential pathophysiological role in thrombogenesis • Inconsistent predictor of CV risk</td>
<td>Decrease</td>
<td>Derosa et al., 2006; Dotsenko et al., 2008; Packard &amp; Libby, 2008(^{81,82,86})</td>
</tr>
<tr>
<td>TNF(_\alpha)</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, plaque rupture, thrombogenesis, ischaemic tissue injury • Predictor of CV risk</td>
<td>Decrease</td>
<td>Dixon &amp; Symmons, 2007; Miyazaki &amp; DeFronzo, 2008; Dotsenko et al., 2008(^{87,88})</td>
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(continued)
TNFα-induced intracellular superoxide radical generation and subsequent expression of the redox-sensitive transcription factor in coronary artery endothelial cells, leading to down-regulation of the LOX-1 gene (LOX-1 is a receptor that facilitates the uptake of ox-LDL).94 Pioglitazone has also been shown to decrease the ox-LDL induced activation of the pro-inflammatory CD40/CD40L system in endothelial cells.97 Studies also show that pioglitazone can improve peripheral vascular endothelial function in patients with type 2 diabetes and coronary endothelial function in patients with CAD.98,99 Animal studies suggest that endothelium-dependent vasodilatation with pioglitazone is mediated via nitric oxide and that improvements in impaired endothelium-dependent relation may involve a reduction in oxidative stress via an increase in super-oxide dismutase (SOD), a suppression of NAD(P)H oxidase activity and a decrease in endothelin-1 level (attributable to an inhibition of the AP-1 signalling pathway).100,101 Recent studies suggest a mechanistic link between the effects of pioglitazone on adiponectin and endothelial vasodilator activity.102 Pioglitazone also increases the number and function of endothelial progenitor cells and their migratory response, thus providing a potential regenerative mechanism in atherosclerosis.103–105 Pioglitazone also regulates mechanisms of endothelial sterol transport at an in vitro blood-brain barrier model without disturbing important barrier properties, which may facilitate efflux of excess cholesterol from the central nervous system.106

VSMCs appear to play an important role in early atherosclerosis via accumulation of plaque lipid, production of extracellular matrix and the release of inflammatory cytokines.107 Pioglitazone inhibits VSMC cell proliferation and migration, as well as increasing VSMC apoptosis.108–111 The effect on migration may relate to the ability of pioglitazone to reduce ox-LDL-stimulated expression of MMP-1 in VSMCs,112 whereas the effect on proliferation may relate to inhibition of connective tissue growth factor expression (CTGF), a factor that stimulates extracellular matrix formation.113 This latter effect has been reported in both advanced atherosclerotic plaques in mice and for TGFβ-stimulated CTGF expression in cultured SMCs.113 The inhibition of VSMC proliferation with pioglitazone is likely to contribute to the slowing of CIMT progression and inhibition of restenosis observed in animal models and clinical studies.114

Several lines of evidence support a role for macrophage-mediated inflammation in the pathogenesis of atherosclerosis.64 In vivo, pioglitazone primes human circulating blood monocytes to differentiate into an anti-inflammatory M2 macrophage phenotype, rather than the pro-inflammatory,

Table 1 Continued

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<th>Relevance to CVD</th>
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<tr>
<td>MCP-1 (aka CCL2)</td>
<td>• Potential pathophysiological role in atherosclerotic lesion formation, plaque rupture, thrombogenesis</td>
<td>Decrease</td>
<td>Pfützer et al., 2005; Aukrust et al., 2008; Forst et al., 2008; Dotsenko et al., 200869,72,81,89</td>
</tr>
<tr>
<td>P-selectin</td>
<td>• Potential pathophysiological role in atherosclerotic lesion progression, thrombogenesis</td>
<td>Decrease</td>
<td>Woollard &amp; Chin-Dusting, 2007; Dotsenko et al., 2008; Forst et al., 200872,81,90</td>
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CCL2 = chemokine (C-C motif) ligand-2; CRP = C-reactive protein; CVD = cardiovascular disease; FFA = free fatty acids; FPG = fasting plasma glucose; HDL = high density lipoprotein; LDL = low density lipoprotein; MCP-1 = monocyte chemoattractant protein-1; MMP-9 = matrix metalloproteinase-9; PAI-1 = plasminogen activator inhibitor-1; sdLDL = small dense LDL; TNFα = tumour necrosis factor-α.

Figure 2. Pioglitazone has direct effects in cells associated with the vasculature. EC = endothelial cell; VSMC = vascular smooth muscle cell. Adapted from Duan et al.92
pro-atherogenic M1 phenotype, both of which are detectable in human atherosclerotic lesions.\textsuperscript{115,116} Pioglitazone also increases the expression of LXR\(\alpha\) and ABCA1 (which facilitate the efflux of cholesterol from macrophages) and reduces cholesteryl ester accumulation (i.e. foam cell formation) in macrophages.\textsuperscript{117}

Insulin insensitivity and hyperinsulinaemia are associated with the promotion of thrombogenesis, so it might be expected that agents that improve insulin sensitivity might also have anti-thrombotic properties.\textsuperscript{118} Accordingly, pioglitazone protects against thrombus formation in animal models,\textsuperscript{119,120} and improves haemostatic variables (PAI-1, sCD40, VWF, FVII:C, FVII:Ag) in humans, including those with type 2 diabetes and/or coronary artery disease.\textsuperscript{72,86,121,122} Furthermore, human platelet activation is also inhibited by pioglitazone.\textsuperscript{123} Such effects may help to counteract the hypercoagulable, pro-aggregatory, pro-thrombotic state that is a characteristic feature of type 2 diabetes.\textsuperscript{124–126}

**Other potential factors**

Fatty liver disease is strongly associated with early carotid atherosclerosis and the presence of CVD independently of classical risk factors, insulin resistance and the presence of the metabolic syndrome.\textsuperscript{127–129} Pioglitazone has been shown to reduce ectopic fat accumulation significantly, particularly with regards to intrahepatic and intramyocellular fat.\textsuperscript{130,131} It remains unclear whether the ability of pioglitazone to reduce liver fat and markers of fatty liver disease in patients with type 2 diabetes or non alcoholic steatohepatitis is relevant to its effects on atherosclerosis and CV events.\textsuperscript{5,129,132,133} A recent study suggests that pioglitazone may reduce myocardial fat accumulation.\textsuperscript{134}

Pioglitazone has a small but significant and consistent beneficial effect on blood pressure and this may be due, at least in part, to a direct vascular effect.\textsuperscript{135,136} This could be particularly relevant for reducing stroke risk, as hypertension represents the most important risk factor for stroke.\textsuperscript{23}

**Beyond prevention of macrovascular events—cardioprotection and neuroprotection**

In addition to prevention of atherothrombogenesis, another aspect of CV protection involves minimizing the ischaemic damage in tissues affected by occlusive vascular events—the myocardium in the case of coronary artery occlusion and neuronal tissue in the case of stroke. Targeting this component of the CVD process may help to decrease the severity of events and/or improve functional recovery. Evidence primarily from animal studies suggests that pioglitazone may have both cardioprotective and neuroprotective properties.

**Cardioprotection**

In animal models, pioglitazone protects against ischaemia/reperfusion injury when administered prior to ischaemia.\textsuperscript{137–140} This cardioprotective effect of pioglitazone has been associated with several factors, including increased activity and expression of phospholipase A\(2\) and COX-2, decreased expression of MCP-1, intercellular adhesion molecule-1 (ICAM-1) and MMP-2, decreased number of infiltrating macrophages, restoration of impaired PI3K–dependent Akt activation and suppression of cardiomyocyte apoptosis.\textsuperscript{137,139–141} Further, the cardioprotective effect of pioglitazone in vivo appears to be additive to that seen with atorvastatin.\textsuperscript{139}

Pioglitazone has also been shown to improve recovery of cardiac function after myocardial ischaemia in rat models.\textsuperscript{142} Unfortunately, these effects on functional recovery after an event have not been investigated in human studies. However, pioglitazone has been shown to improve several aspects of cardiac function in other settings. For instance, pioglitazone was shown to improve LV diastolic function without LV mass regression in hypertensive patients\textsuperscript{143} and to increase the Cardiac Index significantly compared with glibenclamide in patients with type 2 diabetes and systolic dysfunction.\textsuperscript{144} Pioglitazone has also been shown to improve cardiac glucose utilization and blood flow in patients with familial hypercholesterolaemia, suggestive of improved coronary microvascular function.\textsuperscript{145} Recent results from the 24-week PIRAMID study (Pioglitazone Influence on tRiglyceride Accumulation in the Myocardium In Diabetes) suggest that pioglitazone improves myocardial diastolic function and compliance, and increases myocardial glucose uptake (compared with either baseline or metformin comparator therapy) in similarly well-controlled patients with type 2 diabetes without structural heart disease or inducible ischaemia.\textsuperscript{146}

**Neuroprotection**

Pre- or post-treatment with pioglitazone has been shown to decrease infarct volume and neurological deficits significantly in normotensive, normoglycaemic, hypertensive and hyperglycaemic rodent models of stroke.\textsuperscript{147,149} In a more recent animal study, the significant reduction in cerebral infarct size was associated with reduced infarct cell death, reduced gliosis and increased neurotrophic factor levels.\textsuperscript{148}
size with pioglitazone was similar in magnitude to that obtained with the angiotensin receptor blocker candesartan.

This post-ischaemic neuroprotection afforded by pioglitazone has been associated with several factors, including reduced oxidative stress, prevention of COX-2 upregulation and reduced activation of MAP kinases and NF-kB. Pioglitazone also protects against hypertension-induced cerebrovascular injury and stroke independently of blood pressure by improving vascular endothelial dysfunction, inhibiting brain inflammation and reducing oxidative stress. Recent animal data also suggest that pioglitazone can protect neurons against inflammatory stimuli by inhibiting microglial increases in inducible nitric oxide synthase expression and NO production, and this may be mediated by activation of the PI3K/Akt pathway, followed by inhibition of p38 MAP kinase activity. A recent study in rats shows that intracerebral administration of pioglitazone suppresses the expression of IL-6 in ischaemic brain tissue during the initial phase of ischaemic stroke, in which the overproduction of IL-6 may aggravate neuronal damage, but not at later time points, when IL-6 promotes neuroprotection and inhibits neuronal death.

Interestingly, PPARγ expression is upregulated in neurons of the ischaemic penumbra after an ischaemic event in a rodent model and a PPARγ agonist also reduced infarction volume in this model. Ischaemic pre-conditioning also increases PPARγ transcriptional activity based on in vitro models. It has therefore been suggested that PPARγ upregulation may represent an endogenous pro-survival mechanism against ischaemic injury. In line with these observations, increased plasma levels of the endogenous PPARγ agonist 15-deoxy-delta-12,14-prostaglandin J2 are associated with better outcome in ischaemic stroke patients.

None of these potential neuroprotective effects of pioglitazone have been confirmed in human studies. However, some observational evidence suggests that TZDs can enhance functional recovery after stroke in patients with type 2 diabetes. An ongoing placebo-controlled study [the Insulin Resistance Intervention after Stroke (IRIS Trial)] is also looking at the effect of pioglitazone on outcomes in non-diabetic patients with a recent ischaemic stroke or transient ischaemic attack and insulin resistance (http://www.iristrial.org).

Conclusions

Diabetic vascular disease is complex and multifactorial and determining the link between the diverse metabolic effects of pioglitazone and its impact on macrovascular outcomes represents a considerable challenge. So far, evidence strongly suggests that pioglitazone (at current recommended doses) slows the progression of atherosclerosis in different arterial beds (carotid and coronary). Lipid effects are likely to contribute to this effect, and recent data suggest that the ability of pioglitazone to reduce HDL cholesterol might be particularly important. Nevertheless, many other processes may play a role in the protective properties of pioglitazone, such as modulation of the subclinical inflammatory processes thought to underlie atherosclerosis, protection of endothelial function and inhibition of thrombogenesis.

The potential beneficial impact of pioglitazone on cardiovascular risk factors other than hyperglycaemia (e.g. lipids) and macrovascular outcomes needs to be considered within its overall glucose-lowering efficacy and safety profile. Notably, pioglitazone is contraindicated in patients with a history of heart failure (NYHA Class III-IV in the US; Class I-IV in the EU) due to an increased incidence of oedema, which may exacerbate or precipitate the condition. Other important factors that warrant consideration are the tendency to cause weight gain in some patients and a potential increase in fracture risk (primarily in postmenopausal women). On the other hand, its low propensity to cause hypoglycaemia, either as mono- or combination therapy, means that pioglitazone may be a particularly appropriate consideration for patients in whom hypoglycaemia is a concern.

At present, pioglitazone is indicated for the treatment of hyperglycaemia in type 2 diabetes and not specifically for the reduction of atherosclerosis or macrovascular risk. Contemporary diabetes treatment guidelines do, nonetheless, note the potential for pioglitazone to reduce macrovascular risk. One set of contemporary stroke guidelines (from the European Stroke Organization) goes one step further and actively supports the use of pioglitazone as an option in patients with type 2 diabetes (who do not require insulin) after stroke. However, no other guidelines to date have advocated the use of pioglitazone based on macrovascular benefits. Within this context, it may be inappropriate to make any specific recommendations regarding the use of pioglitazone as a cardiovascular drug. Nevertheless, based on the current evidence, it would appear that pioglitazone may be particularly effective at reducing the risk of recurrent events in patients with previous myocardial infarction or stroke. Furthermore, even without considering any beneficial effect on cardiovascular
outcomes, physicians can be relatively confident that pioglitazone is safe from a cardiovascular perspective and it has one of the best-characterized and therefore predictable cardiovascular safety profiles of the currently available oral glucose-lowering agents.\textsuperscript{14,160} Certainly, in terms of cardiac and cerebral macrovascular events, there is absolutely no suggestion of any increased risk, and the excess heart failure risk that occurs in patients on pioglitazone therapy appears to be benign with no adverse impact on macrovascular outcomes or mortality.\textsuperscript{14,160}

Pioglitazone may influence CVD pathophysiology at multiple points in the disease process, including atherogenesis, plaque inflammation, plaque rupture and haemostatic disturbances (i.e. thrombus/embolism formation), as well as microangiopathy. Recent evidence that CV protection may extend further to include protection of the myocardium and cerebral tissue from ischaemic damage during and/or following an event is interesting. However, clinical studies are required to confirm the occurrence of these effects and their contribution to CVD prevention in patients. It is hoped that ongoing studies (including further analyses from completed atherosclerosis imaging studies, such as CHICAGO and PERISCOPE) will help to define further the mechanisms underlying the CV protective effect of pioglitazone. In turn, such analyses could throw open new lines of enquiry in this field and ultimately point the way to new therapeutic opportunities.

Conflict of interest: Professor Erdmann has served as a consultant to, and received travel expenses and payments for speaking at meetings from, and ultimately point the way to new therapeutic opportunities. Takeda. Professor Wilcox has served as a consultant and received travel expenses and payments for speaking at meetings from, as a consultant to, and received travel expenses.

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