EFFECTS OF PPARγ LIGANDS ON ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

C. Fiévet and B. Staels, Institut Pasteur de Lille, Département d’Athérosclérose, Lille, F-59019 France, Inserm, U545, Lille, F-59019 France, Université de Lille 2, Lille, F-59006 France

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors regulating the expression of genes that control lipid and glucose homeostasis, modulating thus the major metabolic disorders predisposing to atherosclerosis and subsequent cardiovascular diseases [1]. Moreover, PPARs exert additional anti-inflammatory and lipid-modulating effects in the arterial wall, being therefore interesting molecular targets for the treatment of atherosclerosis [2]. Finally, PPARs regulate genes that control thrombogenicity which occurs after the rupture of atherosclerotic plaques [3,4]. Three different PPARs have been identified (PPARα, PPARβ/δ, PPARγ) each displaying distinct tissue distribution patterns [5]. PPARs are activated by natural ligands (such as fatty acids and eicosanoids) and by pharmacological agonists. Whereas ligands for PPARβ/δ are still in the experimental stage, PPARα and PPARγ are the targets of two classes of drugs currently used in clinical practice: fibrates and thiazolidinediones (TZDs) respectively. Pioglitazone and rosiglitazone are TZDs currently used for the treatment of type 2 diabetes as insulin sensitizing agents. These drugs have not only significant hypoglycemic effects, but also potential positive effects on lipid metabolism, the endothelium, oxidative stress, and vascular inflammation [6]. These additive actions of TZDs might reduce the development of atherosclerosis.

Preclinical development of PPAR ligands that would act on metabolic factors which enhance atherosclerosis includes the analysis of their effects in suitable murine models. However, the development of a reliable experimental animal model is not easy because ideally the regulatory pathways in these models need to be similar to those in humans [7].

PPARγ and Atherosclerosis in Humans

Whereas the effects of PPARα activation by fibrate treatment on atherogenesis and coronary events in humans are fairly well documented through clinical intervention studies [8], until recently no study data were available answering the questions whether treatment with TZDs translates into a therapeutic benefit in atherosclerotic cardiovascular disease. Most clinical studies performed to date, assessing the effects of TZDs in diabetic patients, suggested vascular protective effects of PPARγ ligands related to improved insulin-sensitivity, decreased vascular and systemic markers of inflammation, reduced carotid wall thickness and neointima formation, and depending on the TZD, corrected dyslipoproteinemia [6]. Moreover, interestingly, even in non-diabetic patients with coronary artery disease, rosiglitazone treatment induced beneficial effects on the vascular endothelium, exerted anti-platelet activities, and reduced carotid intima-media thickness progression [9-13]. All these reports argued for potent anti-atherogenic effects of TZDs, raising moreover the possibility of their additional use in clinical conditions other than diabetes. The PROactive study [14] is a prospective trial addressing the role of the PPARγ activator pioglitazone in the prevention of macrovascular events in patients with type 2 diabetes and pre-existing cardiovascular disease. The results of this study were just reported [15]. Although the primary endpoint, which was composed of a combination of disease-related
(mortality, non-fatal myocardial infarction, stroke, and acute coronary syndrome) and procedural (coronary and leg revascularisation and leg amputation) endpoints, did not reach significance, pioglitazone treatment significantly reduced the principal secondary endpoint (all cause mortality, non-fatal myocardial infarction, and stroke) by 16%. Moreover, the results from a first post-hoc analysis, presented at the recent AHA scientific sessions in Dallas, in the subgroup of patients with prior myocardial infarction showed that pioglitazone decreased recurrent cardiovascular events by 19%, fatal and non-fatal myocardial infarction by 28%, and acute coronary syndrome by 37% (www.proactive-results.com). Altogether, treatment with pioglitazone did not result in major adverse events, with the exception of an increase of reported, non-adjudicated heart failure. Since death due to heart failure was similar in the placebo and pioglitazone groups, it is likely that the high reported number in the pioglitazone group is due to the well-known increase in edema and thus does not represent bona fide heart failure. These results thus further indicate that pioglitazone treatment, on top of optimal cardiovascular treatment, reduces cardiovascular risk in type 2 diabetics with prior cardiovascular disease. The next trial in type 2 diabetic patients to report is the RECORD study with rosiglitazone which is still running [16].

**PPARγ and Mouse Models of Atherosclerosis**

Previous studies performed in different mouse models (LDL receptor- or apo E-deficient mice) all demonstrated an atheroprotective effect of PPARγ activation by TZDs [17]. From these studies, it could be plausibly concluded that the overall beneficial action of the TZDs occurred independently from systemic lipid changes, and, possibly, from the insulin sensitizer activity of the compounds. Recently, we investigated the effect of two different PPARγ ligands (rosiglitazone and pioglitazone) on atherogenesis in a non-diabetic murine model which displays mixed dyslipidemia and develops atherosclerotic lesions consisting mainly of foam cells, i.e. homozygous human apolipoprotein (apo) E2 knock-in mice (E2-KI mice) that express human apoE2 instead of mouse apoE [18]. Surprisingly, when using this model, we demonstrated that PPARγ ligands did not protect E2-KI mice from atherosclerosis and foam cell accumulation. Also no significant changes in lipoprotein metabolism were observed in rosiglitazone- or pioglitazone-treated mice. Although glucose homeostasis was improved in the mice, this improvement was not sufficient to induce a delay in atherosclerosis development in this mouse model of normal glucose homeostasis.

PPARγ is expressed in all cell types of the arterial wall, including endothelial cells, monocytes/macrophages and vascular smooth muscle cells (SMCs) [2] and besides its activity on macrophage lipid homeostasis with direct consequences for atherosclerosis development [19], PPARγ modulates the earliest step of the atherosclerotic lesion by inhibiting the expression of certain cytokines involved in the recruitment of monocytes/macrophages by endothelial cells [20]. Through these properties, PPARγ may exert cardiopreventive activities by decreasing foam cell formation. In our study in E2-KI mice, the lack of effect of PPARγ activation on macrophage accumulation contrasts with previous in vivo animal studies showing protective effects [17]. Although the exact reasons are unclear, several explanations can be evoked. Atherosclerosis in the E2-KI mice, in contrast to other mouse models, is characterized by the almost exclusive presence of macrophages. It is possible that, in the context of severe, uncorrected dyslipidemia, PPARγ activation in macrophages is insufficient to reverse the proatherogenic program. Moreover, a substantial controversy exists on the role of PPARγ in
macrophage cholesterol metabolism in mice. *In vitro*, PPARγ ligands may induce macrophage foam cell formation through CD36 induction and increased uptake of oxidized LDL [21]. *In vivo* rosiglitazone treatment in obese insulin-resistant ob/ob mice resulted in a decrease of peritoneal macrophage CD36 protein content likely due to the insulin sensitizing activity of the compound [22]. However, LDL receptor-deficient mice that display only mild insulin-resistance showed an increase in CD36 protein after rosiglitazone treatment *in vivo* [22]. One can note the pro-atherogenic role of the scavenger receptor CD36 has been recently challenged [23], nevertheless we can suggest that the effects of rosiglitazone on foam cell formation and atherogenesis could differ depending on the degree of insulin resistance. E2-KI mice are non-diabetic and are not insulin resistant, and therefore the impact of PPARγ agonists on macrophages may not be sufficient to improve atherosclerosis in this mouse model.

Whether these observations can be extended to the human situation is unclear. Previous results on human and murine macrophages have demonstrated significant species-differences in the control of cholesterol homeostasis by PPARγ ligands [19,20]. For instance, whereas PPARγ activation results in the induction of the cholesterol efflux transporter ABCA1 in human macrophages [19], this effect is much less pronounced in murine macrophages [20]. Finally, it is possible that the atheroprotective effects of PPARγ are mediated via other cell-types that do not participate in atherogenesis in E2-KI mice such as SMCs. In this respect, it is interesting to note that TZD treatment has been shown to improve endothelial function and decrease intima-media thickness also in non-diabetic patients [9-13]. Such activities may be mediated, in part, via their effects on the proliferation and migration of vascular SMCs and the anti-inflammatory activities in these and other cell types involved in atherogenesis [24,25]. Moreover, PPARα and PPARγ ligands may influence thrombogenesis and inhibit proteins implicated in plaque rupture resulting in plaque stabilization [3,4]. Therefore, we cannot exclude that determining the effects of TZDs on advanced atherosclerosis may prove to be a stronger predictor of their potential clinical benefit and further testing their effects in other atherosclerosis models and in humans is therefore warranted.

**References**


Address correspondence to:
Catherine Fiévet