Losartan Metabolite EXP3179
An AT1-Receptor–Independent Treatment Strategy for Patients With the Metabolic Syndrome?

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Losartan potassium, the first orally active nonpeptide angiotensin receptor blocker (ARB) to be introduced for clinical use in hypertension, is rapidly absorbed after oral administration, reaching a peak in plasma 1 to 2 hours. Despite being metabolized by the cytochrome P450 (CYP) 3A4, 2C9, and 2C10 isoenzymes, losartan has no clinically relevant interactions with inhibitors and stimulators of the CYP450 system and, like the other ARBs, is devoid of significant adverse effects: hypotension-related dizziness was the only drug-related event reported more frequently with losartan monotherapy than with placebo in clinical trials.1 After oral administration about 14% of a losartan dose is converted to the EXP3174 metabolite, which is 10- to 40-fold more potent than losartan and mediates most of its angiotensin type 1 receptor (AT1-R)–blocking effects. Losartan was generally deemed to be devoid of agonistic properties,2 but an important intermediate aldehyde metabolite EXP3179 has been identified (Figure).3 EXP3179 has no AT1-R–blocking activity, but potently inhibits the expression of endothelial cyclooxygenase (COX)-2, thereby exerting potent antiinflammatory actions. It also blocked intercellular adhesion molecule (ICAM)-1 mRNA upregulation and COX-dependent thromboxane A2 and prostaglandine F2α generation in vitro. Moreover, EXP3179 stimulated the phosphorylation of the endothelial nitric oxide synthase through a PI3-kinase/Akt pathway downstream of the VEGF-receptor 2 in cultured endothelial cells.4 As the ligand-activated PPAR-γ also exerts antiinflammatory actions (by inhibiting the action of proinflammatory transcription factors, such as AP-1 and nuclear factor κB), and represses COX-2 promoter activity and mRNA expression (by interacting with the c-Jun component of the AP-1 complex), in this issue of Hypertension Kappert et al investigated the hypothesis that EXP3179-mediated activation of PPAR-γ entails a novel mechanism of the antiinflammatory antidiabetic actions of losartan.5

PPAR-γ as a Therapeutic Target
PPAR-γ is a nuclear transcription factor that exists in the form of a heterodimer complex with the retinoid X receptor–α.6 On PPAR-γ activation the receptor complex triggers the expression of key target genes that mediate beneficial effects on glucose and lipid metabolism. Studies of individuals with mutated forms of the receptor furnished compelling evidence for the importance of PPAR-γ activity in ameliorating the MS and diabetes.7 Such individuals with a loss-of-function mutation exhibit the features of the MS, including severe insulin resistance, hypertriglyceridemia, elevated concentrations of nonesterified fatty acids, low HDL cholesterol concentrations, and hypertension. Hence, PPAR-γ is currently regarded as a therapeutic target in the MS. The PPAR-γ activators thiazolidinediones, pioglitazone, and rosiglitazone are currently available and have been shown to increase insulin sensitivity and decrease fatty acids and triglycerides concentration in type 2 DM patients.8

The fact that PPAR-γ is abundantly expressed also in vascular and nonvascular cells (monocytes/macrophages, endothelial cells, vascular smooth muscle cells) in the vessel wall lends further support to exploit use of ligand-activated PPAR-γ as antiatherosclerotic strategy with the ultimate goal of lowering cardiovascular morbidity and mortality.9 Therefore, the hypothesis put forward by Kappert et al that losartan can act as a PPAR-γ activator via EXP3179 is sound.10

ARBS and Losartan as PPAR-γ Agonists
Some ARBs, including telmisartan, irbesartan, and losartan, were shown to have some PPAR-γ activity,11 with a potency that paralleled their lipophilicity, telmisartan being more potent than irbesartan. Schupp et al showed that losartan enhanced PPAR-γ–dependent adipocyte differentiation and the expression of the adipogenic marker gene adipose protein 2 (aP2) gene, whereas other ARBs, including eprosartan and valsartan, were ineffective.12 Because losartan exerted this effect only at very high concentrations (100 μmol/L) that are not achieved in vivo, it seems unlikely that it can activate PPAR-γ per se. By contrast EXP3179 was more potent than losartan in inducing PPAR-γ activation, suggesting that it mediates the antidiabetic properties of losartan. PPAR-γ–mediated adipocyte differentiation, as well as activation of the PPAR-γ ligand binding domain (LBD) were reported to occur at EXP3179 concentrations (1 to 10 μmol/L), which are not far above the maximum serum concentrations of EXP3179 (between 0.1 and 1 μmol/L) that were achieved after a single oral dose of losartan (100 mg).13 Moreover, being lipophilic, EXP3179 can attain sufficient intracellular concentrations to exert its PPAR-γ effects in vivo.

Schupp et al also showed that of the 2 main metabolites of losartan (Figure), EXP3174 and EXP3179, the former is the main antihypertensive AT1-R–blocking metabolite, whereas the latter showed PPAR-γ–activating properties. Noteworthy,
Maximum response induced by the full PPAR-LBD activation by EXP3179 corresponded to 51% of the upregulated by 3.75-fold. They found that CD36 was reaching a maximum at 100 s in patients not receiving any ARBs. They found detectable plasma levels of Exp-3179 and Exp-3174 in 15 hypertensive patients chronically treated with losartan and in 7 hypertensive patients with and without chronic losartan, as well as losartan and EXP3174, did induce CD36 gene expression in primary human monocytes in vitro at the concentrations found in hypertensive patients chronically treated with losartan. The EXP3179 effect was mimicked by telmisartan and irbesartan treatment, underlining the dual molecular targets of these ARBs.

In this issue of Hypertension Kappert et al investigated the plasma levels of Exp-3179 and Exp-3174 in 15 hypertensive patients chronically treated with losartan and in 7 hypertensive patients not receiving any ARBs. They found detectable serum levels of EXP3179, which peaked after 2 hours at 1.92 μmol/L. As PPAR-γ activators were described to enhance the expression of the scavenger receptor CD36 and the cholesterol efflux transporter ABCG1, the authors measured the expression of these genes in monocytes isolated from their patients, taken as a model for in vivo PPAR-γ-activated target gene expression. They found that CD36 was upregulated by 3.75-fold ($P=0.043$) and ABCG1×252-fold ($P=0.0045$) in patients chronically treated with losartan compared to losartan-untreated patients. As angiotensin II was shown to reduce cholesterol efflux from monocytes/macrophages, and both these genes have been implicated in cholesterol uptake and removal in monocytes/macrophages and therefore in atherogenesis through foam cell formation, these results can functionally be important.

To prove the functional relevance of PPAR-γ agonism, Kappert et al also analyzed in vitro the cellular responses of human monocytes to monocyte chemoattractant protein-1 (MCP-1)-directed chemotaxis in the absence or presence of pioglitazone and of ARBs known to activate PPAR-γ (telmisartan, irbesartan, losartan, and its metabolites EXP3174 and EXP3179, or valsartan, an ARB without PPAR-γ agonistic properties. They found that MCP-1–directed migration was concentration-dependently impaired by the molecules with (EXP3179, telmisartan, irbesartan, and pioglitazone) but not by those without (losartan, EXP3174, and valsartan) PPAR-γ activity. To support their ex vivo findings on steady state mRNA, they also showed that EXP3179, but not losartan and EXP3174, did induce CD36 gene expression in primary human monocytes at the concentrations found in hypertensive patients chronically treated with losartan. The EXP3179 effect was mimicked by telmisartan and irbesartan treatment, underlining the dual molecular targets of these ARBs.

Hence, Kappert et al concluded that in vivo under chronic treatment with losartan the levels of EXP3179 were sufficient to induce PPAR-γ target gene expression and to elicit functional effects that can be beneficial in terms of improving the MS and preventing atherosclerosis.

**Limitations of the Study**

These results can be clinically relevant, and the authors should also be commended for developing a technique to investigate PPAR-γ activation ex vivo. However, there are caveats that should be noticed. The study is small, open-label, nonrandomized, nonplacebo controlled. The phenotypic characterization of the hypertensive patients is scant, and it would be interesting to know at least their renin and aldosterone profile, as it is conceivable that the losartan metabolites effect can vary according to the degree of activation of the RAS. Furthermore, there were several differences between the hypertensive patients with and without chronic losartan, which relate to age, BMI, coronary artery disease, ongoing treatment, and prevalence of diabetes. These differences theoretically could have been differentially influenced losartan or EXP metabolism between the treatment groups. A within-patient study design would certainly be more reassuring in showing that the observed gene expression differences were unrelated to the effect of potential confounders and therefore in allowing the generalization of these findings.

The kinetics of EXP3179 likely requires further studies: the EXP3179 concentration appears to be a short-lived. It attained peak at 2 hours after losartan administration, after which there was an abrupt tail-off suggesting extensive hepatic metabolism. By contrast, the EXP3174 levels, and therefore AT1-R blockade, would seem to be maintained or even increased when the EXP3179 levels were markedly reduced. The transient increase in EXP3179 suggests that...
plasma levels in the range required for PPAR-γ (1 to 10 μmol/L) are reached only briefly in vivo after dosing.

Finally, the method used for the drug measurement apparently does not allow distinguishing between free and protein-bound parental drug and metabolites. This is a relevant issue inasmuch as losartan and EXP-1374 are highly bound to plasma protein (>98%), and EXP3179 is highly lipophilic and therefore also likely to be bound to plasma lipids. The high lipophilicity could improve EXP3179 accumulation in target cells, thus producing sufficient EXP3179 intracellular concentrations in vivo to activate PPAR-γ. However, this might also imply that the free drug concentration can be much lower, possibly unable to activate PPAR-γ, than those actually measured in serum.

Conclusions and Perspectives
AT1-R blockade and PPAR-γ activation have been shown to exert beneficial actions on cardiovascular morbidity and mortality. The possibility of hitting with one drug in a synergistic way the multiple pathogenic targets that concur to increase the global risk profile in the multitude of hypertensive patients with the MS appears much appealing. This, not only because of the clustering of risk factors in hypertensive patients, but also because patient’s adherence to lifestyle modification and multiple drug treatment regimen is often disappointingly poor and generally inversely related to the number of pills to be taken daily. In the long run the possibility of achieving insulin-sensitizing/antidiabetic effects is particularly attractive given the increasing risk of developing DM with aging. The study by Kappert et al demonstrates significant monocyte PPAR-γ target gene regulation by chronic treatment with losartan, which likely is triggered by its metabolite EXP3179. Even though “dual” drugs have not had a blessed destiny in hypertension thus far, this study can be a first step along the right direction to further reduce the still exceedingly high rate of cardiovascular events associated with the MS. The fact that nonhypotensive doses of ARBs were recently shown to prevent cognitive decline partly via PPAR-γ activity in a mouse model of Alzheimer disease suggests that PPAR-γ activity might confer an additional “plus” in the long-term treatment of hypertension.16

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None.

References