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Activation of Liver X Receptors Promotes Neuroprotection and Reduces Brain Inflammation in Experimental Stroke

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Background—The liver X receptors (LXRs) belong to the nuclear receptor superfamily and act as transcriptional regulators of cholesterol metabolism in several tissues. Recent work also has identified LXRs as potent antiinflammatory molecules in macrophages and other immune cells. Combined changes in lipid and inflammatory profiles are likely mediating the protective role of LXRs in models of chronic injury like atherosclerosis. These beneficial actions, however, have not been illustrated in other models of acute injury such as stroke in which inflammation is an important pathophysiological feature.

Methods and Results—We have studied LXR expression and function in the course of experimental stroke caused by permanent middle cerebral artery occlusion in rats and mice. Here, we show that administration of the synthetic LXR agonists GW3965 or TO901317 after the ischemic occlusion improves stroke outcome as shown by decreased infarct volume area and better neurological scores in rats. Neuroprotection observed with LXR agonists correlated with decreased expression of proinflammatory genes in the brain and with reduced nuclear factor-κB transcriptional activity. Loss of function studies using $LXR\alpha,\beta^{-/-}$ mice demonstrated that the effect of LXR agonists is receptor specific. Interestingly, infarcted brain area and inflammatory signaling were significantly extended in $LXR\alpha,\beta^{-/-}$ mice compared with control animals, indicating that endogenous LXR signaling mediates neuroprotection in this setting.

Conclusion—This work highlights the transcriptional action of LXR as a protective pathway in brain injury and the potential use of LXR agonists as therapeutic agents in stroke. (***Circulation***. 2008;118:1450-1459.)

Key Words: cerebral ischemia ■ inflammation ■ nervous system ■ nuclear receptors

Liver X receptors (LXRs) α and β (LXRβ), also known as *NR1H3* and *NR1H2*, respectively, are ligand-activated transcription factors that belong to the nuclear receptor superfamily. Whereas LXRα is expressed predominantly in liver, kidney, intestine, and tissue macrophages, LXRβ is expressed ubiquitously.[1,2] LXRs are activated by certain cholesterol derivatives such as several oxidized cholesterol metabolites or oxysterols. LXRs activate gene expression through binding to promoter regions containing specific hexamer repeats (DR4 elements or LXRE) in association with the obligatory heterodimer partner, the retinoid X receptor, and thus regulate the expression of a number of genes involved in cholesterol metabolism.[2,3] In addition, LXRs antagonize the expression of inflammatory genes activated by microbial components or proinflammatory cytokines in macrophages[4,5] such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), several interleukins (IL-6, IL-1β), and matrix metalloproteinases (MMPs). The antiinflammatory actions of LXR agonists are observed in macrophages from either LXR$\alpha^{-/-}$ or LXR$\beta^{-/-}$ mice but not in macrophages lacking both LXR isoforms (LXR$\alpha,\beta^{-/-}$), suggesting that both receptors can repress inflammatory gene expression in a ligand-dependent manner. It is likely that LXR-dependent antiinflammatory properties are mediated by interaction of LXR with different factors involved in inflammatory gene expression such as the nuclear factor-κB (NF-κB) and small ubiquitin-related modifier (SUMO) pathways.[4,6,7] These actions have been observed not only in macrophages but also in other settings, including the central nervous system; indeed, LXR agonists inhibit lipopolysaccharide-induced inflammatory responses in isolated microglia and astrocytes[8–10] and play a protective role in experimental autoimmune encephalomyelitis.[11] Apart from these antiinflammatory actions, LXRs control the expression of several genes important for cholesterol homeostasis in the brain,[12,13] and LXR agonists reduce amyloid β-peptide formation[14–16] in neural cells. All these actions may explain why LXR agonists are considered useful therapeutic tools in neurodegenerative

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situations associated with dysfunction of lipid metabolism such as Alzheimer's disease[17,18] and motor neuron degeneration[19,20] or in metabolic disorders with brain degeneration such as Niemann-Pick disease.[21]

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All these pieces of evidence suggest that LXR activation could exert a protective role in other central nervous system pathologies in which inflammation is involved such as stroke. Therefore, we explored whether activation of LXR with synthetic agonists causes neuroprotective effects in experimental stroke in rats. We have used 2 structurally unrelated, synthetic nonsteroidal LXR agonists: GW3965, with EC_{50} from 30 to 190 nmol/L in different cell-based reporter gene assays,[22] and TO901317,[23] with an EC_{50} of ≈ 50 nmol/L. We have explored further the role of endogenous LXR signaling during stroke with loss of function studies by using LXR$\alpha,\beta^{-/-}$ mice.

Methods

Materials

GW3965 (3-[3-[N-(2-chloro-3-trifluoromethylbenzyl)-(2,2-diphenylethyl) amino]propyloxy] phenylacetic acid hydrochloride) was kindly donated by Jon Collins (GlaxoSmithKline, Research Triangle Park, NC), and TO901317 (N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide) was from Calbiochem (Merck Chemicals Ltd, Nottingham, UK). The rest of the reagents were from Sigma (Madrid, Spain) or as indicated.

Animals

Adult male Fischer rats (average weight, 225 to 250 g) were used. In addition, wild-type controls and Nr1h3$^{-/-}$Nr1h2$^{-/-}$ double-mutant (LXR$\alpha,\beta^{-/-}$) mice (average weight, 25 to 30 g) on a Sv129/C57BL/6 background were obtained through a collaboration with Drs David Mangelsdorf and Peter Tontonoz. All experimental protocols adhered to the guidelines of the Animal Welfare Committee of the Universidad Complutense (EU directives 86/609/CEE and 2003/65/CE).

Middle Cerebral Artery Occlusion

All experiments were performed in a randomized fashion by investigators blinded to treatment groups. Permanent focal cerebral ischemia was induced by occlusion of the ipsilateral middle cerebral artery (MCAO) by cauterization as described.[24,25] Rats/mice in which the MCA was exposed but not occluded served as sham-operated controls (sham). After surgery, individual animals were returned to their cages with free access to water and food.

Experimental Groups

Several groups were used for determinations of infarct outcome in rats: MCAO followed 10 minutes later by an intraperitoneal injection of saline (n=8); dimethyl sulfoxide (vehicle; 10% in saline; n=10); 10, 20, and 50 mg/kg GW3965 (n=8 to 10); or 10, 20, and 50 mg/kg TO901317 (n=8 to 10). Sham-operated animals received an intraperitoneal injection of saline 10 minutes after the occlusion (sham; n=10). Another group of MCAO-exposed rats received either vehicle or 20 mg/kg GW3965 1 hour after the occlusion (n=8). Injection volume was 0.25 mL/250 g body weight. Other experiments were designed for infarct outcome determination in LXR$\alpha,\beta^{-/-}$ mice and their wild-type littermates (n=8). Additional groups for molecular determinations were used as indicated.

Infarct Size

Infarct size was determined 48 hours after MCAO as described.[24,25] To exclude the brain edema effects, infarct area was corrected by the ratio of the entire area of the ipsilateral hemisphere to that of the contralateral. Infarct volume was calculated as an orthogonal projection. Infarct areas were represented according to their distance from the point of juncture of the coronal and sagittal skull sutures or bregma.

Neurological Characterization

Before death, sensorimotor performance was evaluated with a neurological deficit score.[26] For mice, an additional evaluation was performed with the grip test.[27] Weight loss from status before MCAO to that 48 hours after MCAO was assessed and represented as percent of the initial value. Two independent observers blinded to experimental procedure evaluated neurological characterization.

Protein Expression in Brain Homogenates and Nuclear Extracts

Brain tissue was collected from the peri-infarct areas at different times. For determination of inhibitor κBα (iκBα), iNOS, COX-2, and MMP-9, rats (n=6) were killed 18 hours after MCAO. For determination of ATP-binding cassette transporter (ABCA1) and LXRs, rats were killed 48 hours after MCAO (n=6). Homogenates were prepared as described.[24,25]

For determination of the nuclear NF-κB subunit p65, rats (n=6) were killed 90 minutes after MCAO. Nuclear extracts were prepared as described.[28]

Western Blot Analysis

Western blot was performed as described.[24,25] Incubation was performed with specific primary antibodies against LXRα and LXRβ (ABCAM, Cambridge, UK; 1:1000), p65 (Santa Cruz Technology, Santa Cruz, Calif; 1:1000), IκBα (Santa Cruz, 1:1000), iNOS (Santa Cruz; 1:500), COX-2 (Santa Cruz, 1:1000), MMP-9 (Chemicon, Temecula, Calif; 1:2000), and ABCA1 (ABCAM, 1:1000). β-Actin and Sp1 levels were used as loading controls for total and nuclear protein expression, respectively.

### Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from peri-infarct areas of brains of mice (n=6, killed 8 hours after MCAO) using Trizol reagent (Invitrogen, Barcelona, Spain). RNA (1 μg) was reverse transcribed with iScript cDNA Synthesis kit (Bio-Rad Laboratories, Alcobendas, Madrid, Spain). Quantitative real-time polymerase chain reaction was performed with a Bio-Rad iQ5 Thermocycler with triplicate samples and normalized to 36B4 levels. Specific primers for mouse genes were designed using Primer Express software (Applied Biosystems, Alcobendas, Madrid, Spain) and are as follows: mABCA1 (forward, GGTTTGGAGATGGTTATACAATAGTTGT; reverse, CCCG-GAAACGCAAGTCC), mSREBP1c (forward, GGAGCCATGGAT-TGCACATT; reverse, GGCCCGGGAAGTCACTGT), mIL6 (forward, CCAGGTAGCTATGGTACTCCAGAA; reverse, GC-TACCAAACTGGATATAATCAGGA), mIL12p40 (forward, TT-GCTGGTGTCTCCACTCAT; reverse, GGGAGTCCAGTCCAC-CTCTA), mouse regulated on activation, normal T cell expressed and secreted (mRANTES) (forward, GTGCCCACGTCAAGGAG-TAT; reverse, CCCACTTCTTCTCTGGGTTG), mMCP1 (forward, GATCATCTTGCTGGTGAATGAGT; reverse, CATCCACGTGT-TGGCTCA), and m36B4 (forward, AGATGCAGCAGATCCG-CAT; reverse, GTTCTTGCCCATCAGCACC).

Brain Concentrations of IL-1β and Tumor Necrosis Factor-α

Supernatants from brain homogenates were used for determinations with a commercially available kit (Biotrak ELISA System, GE-Healthcare, Barcelona, Spain).

Statistical Analysis

Results are expressed as mean$\pm$SEM of the indicated number of experiments; statistical analysis involved 1-way ANOVA (or the
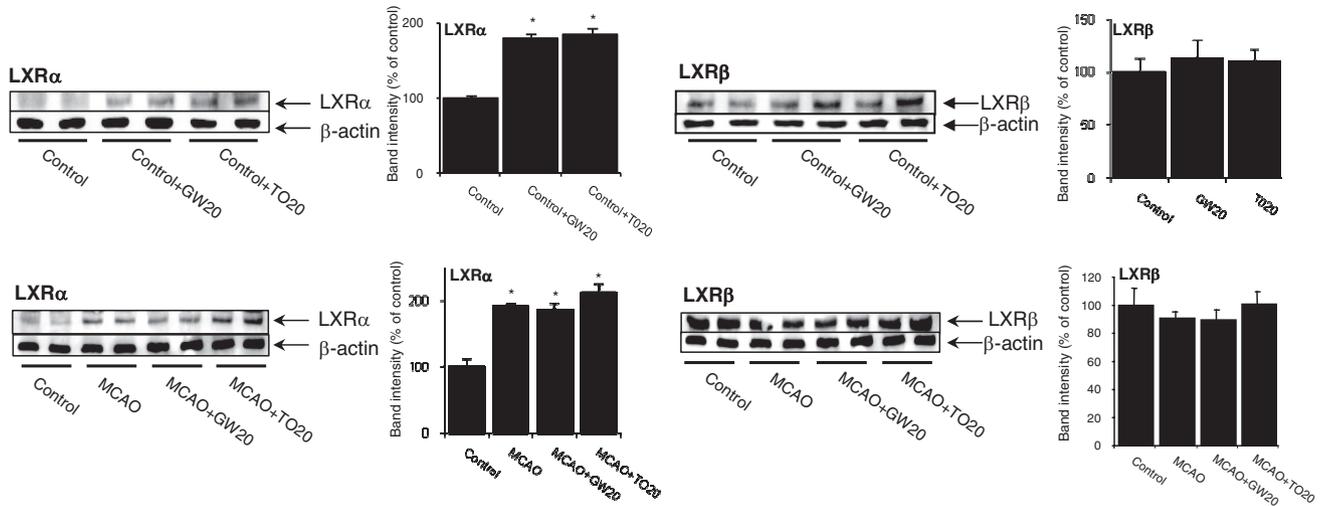
Figure 1. Expression of LXRα and LXRαβ receptor isoforms in rat brain. Effect of the LXR agonists GW3965 (20 mg/kg; GW20) or TO901317 (20 mg/kg; TO20) in control and experimental stroke (MCAO)–exposed animals. Western blot analysis shows a representative blot from brain homogenates; right panels show the densitometric analysis of bands from all blots. Data are mean±SEM; n=6; *$P<0.05$ vs control.

Mann–Whitney test when the data were not normally distributed), followed by individual comparisons of means (Student-Newman-Keuls or Dunn's method when the data were not normally distributed). Values of $P<0.05$ were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

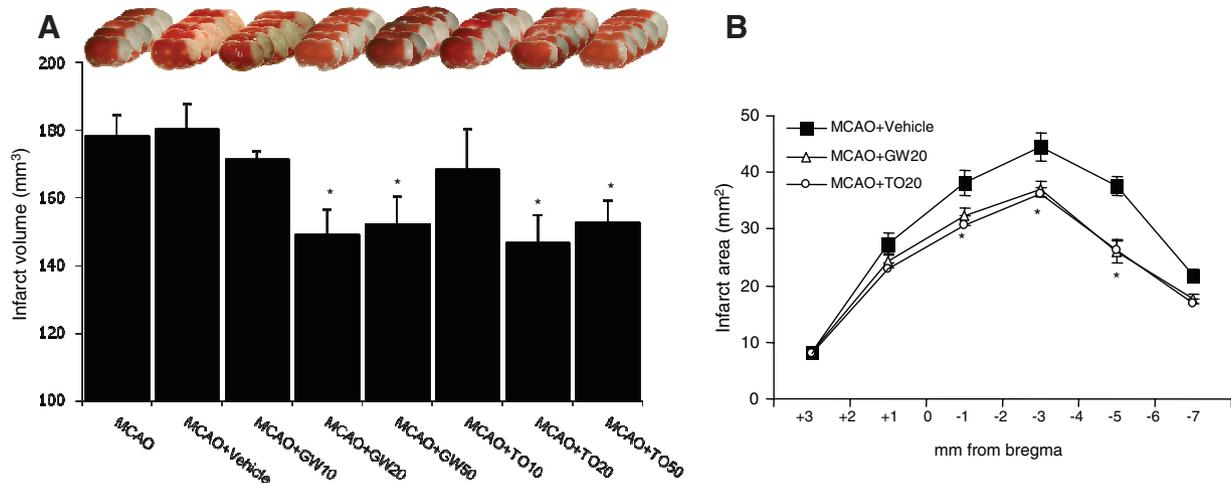### LXR Agonists Are Protective in Experimental Stroke: Effects on Endogenous Gene Expression

Western blot protein analysis showed that LXRβ was robustly expressed in brain tissue of control rats, whereas LXRα levels were significantly lower (Figure 1). Exposure to

MCAO resulted in a significant increase in LXRα protein expression in the peri-infarct area after 48 hours, whereas LXRβ expression remained mostly unaffected. Protein levels of the LXRα but not the LXRβ isoform were increased by treatment with GW3965 or TO901317 in sham but not significantly in MCAO-exposed animals (Figure 1). Results were unaffected by higher doses of LXR agonists (data not shown).

We also evaluated the effect of both GW3965 and TO901317 agonists on a well-known LXR target gene, the transporter ABCA1,[29,30] in rat brain extracts. ABCA1 expression was not affected in MCAO-exposed animals but was potently induced by administration of GW3965 (20 mg/kg) or
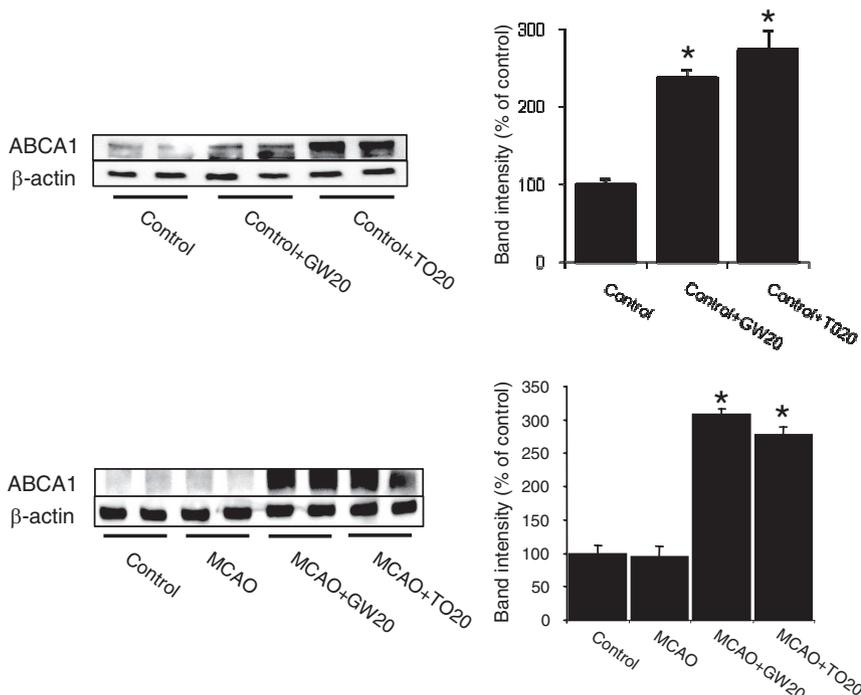
Figure 2. Brain levels of the ABCA-1, an LXR target gene. ABCA1 expression levels were determined in control and ischemic (MCAO) brains after administration of vehicle, GW3965 (20 mg/kg; GW20), or TO901317 (20 mg/kg; TO20). Western blot analysis shows a representative blot from brain homogenates; the right panel shows the densitometric analysis of bands from all blots. Data are mean±SEM; n=6; *$P<0.05$ vs MCAO.

Figure 3. Neuroprotective effect of the LXR agonists in experimental stroke. GW3965 (10, 20, 50 mg/kg; MCAO+GW10/20/50) or TO901317 (10, 20, 50 mg/kg; MCAO+TO10/20/50) reduced infarct volume (A) and infarct areas (B) after permanent MCAO. Data are mean±SEM; n=8 to 10; *$P<0.05$ vs MCAO (see Methods for details). Photographs are of brain slices from representative experiments.

TO901317 (20 mg/kg) in the peri-infarct area, demonstrating that brain LXRs are being activated in a ligand-dependent manner after MCAO (Figure 2).

Therefore, we analyzed the infarct size after 48 hours in rats that received a systemic administration of LXR synthetic agonists after MCAO. First, no spontaneous mortality was found in the MCAO group, a result unaffected by LXR agonists. Activation of LXR by GW3965 or TO901317 (20 to 50 mg/kg; Figure 3) 10 minutes after the occlusion resulted in a significant decrease in MCAO-induced infarct size. Both ligands at 20 to 50 mg/kg, but not at 10 mg/kg, reduced MCAO-induced injury with equivalent efficacy. Importantly, similar effects were obtained when these drugs were administered as late as 1 hour after the occlusion (143.41±8.85 or 145.46±10.21 mm^3 after 20 mg/kg GW3965 or 20 mg/kg TO901317, respectively, versus 180.43±7.67 mm^3 in MCAO plus vehicle; n=8 to 10; $P<0.05$).

Rats treated with LXR synthetic agonists showed better scores in a neurological assessment scale after MCAO (Table 1). Furthermore, MCAO-induced weight loss was lower in those rats receiving LXR agonists (10.47±0.36% in control versus 8.01±0.76% and 7.70±0.72% after 20 mg/kg GW3965 and TO901317, respectively; n=8 to 10; $P<0.05$).

Effect of LXR Agonists on Ischemia-Induced Inflammatory Gene Expression and NF-κB Transcriptional Activity

Next, we analyzed the expression of proinflammatory markers in brain homogenates from peri-infarct tissue of MCAO-injured rats. Acute expression of inflammatory mediators such as iNOS, COX-2, and MMP-9 and proinflammatory

cytokines, including tumor necrosis factor-α (TNF-α) and IL-1β (reviewed elsewhere[31]), participates in brain damage after stroke. MCAO resulted in potent induction of iNOS and COX-2, as shown by the levels found 18 hours after MCAO (Figure 4). Administration of GW3965 or TO901317 inhibited MCAO-induced expression of iNOS and COX-2 levels at the time examined (Figure 4).

MMP-9 mediates damage in cerebral ischemia.[32] MCAO caused an increase in the levels of mature MMP-9 and its precursor pro-MMP-9 (Figure 4). The LXR agonists GW3965 and TO901317 decreased the levels of both forms of this metalloproteinase after MCAO (Figure 4).

MCAO resulted in brain accumulation of IL-1β and TNF-α 18 hours after the ischemic insult. Administration of the LXR agonists GW3965 and TO901317 decreased MCAO-induced expression of IL-1β but not of TNF-α (Figure 5). The LXR agonists did not modify the levels of all these mediators in animals not exposed to MCAO (data not shown).

NF-κB is a transcription factor with a key role in the expression of a variety of genes involved in inflammatory responses.[33] As a sign of its activation, the nuclear levels of its subunit p65 were determined 90 minutes after MCAO. Experimental ischemia caused activation of NF-κB as revealed by the nuclear translocation of p65, as well as an increase in the late levels (18 hours after MCAO) of IκBα, an indicator of an increase in NF-κB transcriptional activity.[34,35] As expected, the LXR agonists GW3965 and TO901317 (20 mg/kg) did not modify p65 nuclear levels after MCAO (Figure 6) but decreased the levels of the NF-κB target gene IκBα.

Table 1. Neurological Status After MCAO in Rats: Effect of LXR Agonists

	MCAO	MCAO+Vehicle	MCAO+20 mg/kg GW3965	MCAO+50 mg/kg GW3965	MCAO+20 mg/kg TO901317	MCAO+50 mg/kg TO901317
Neurological deficit score	3.20±0.21	3.10±0.24	2.60±0.17*	2.70±0.27*	2.10±0.19*	1.90±0.19*

The LXR agonists GW3965 and TO901317 improved the neurological status of rats exposed to MCAO. Data are shown as mean±SEM.
*$P<0.05$ vs MCAO+vehicle (n=10).
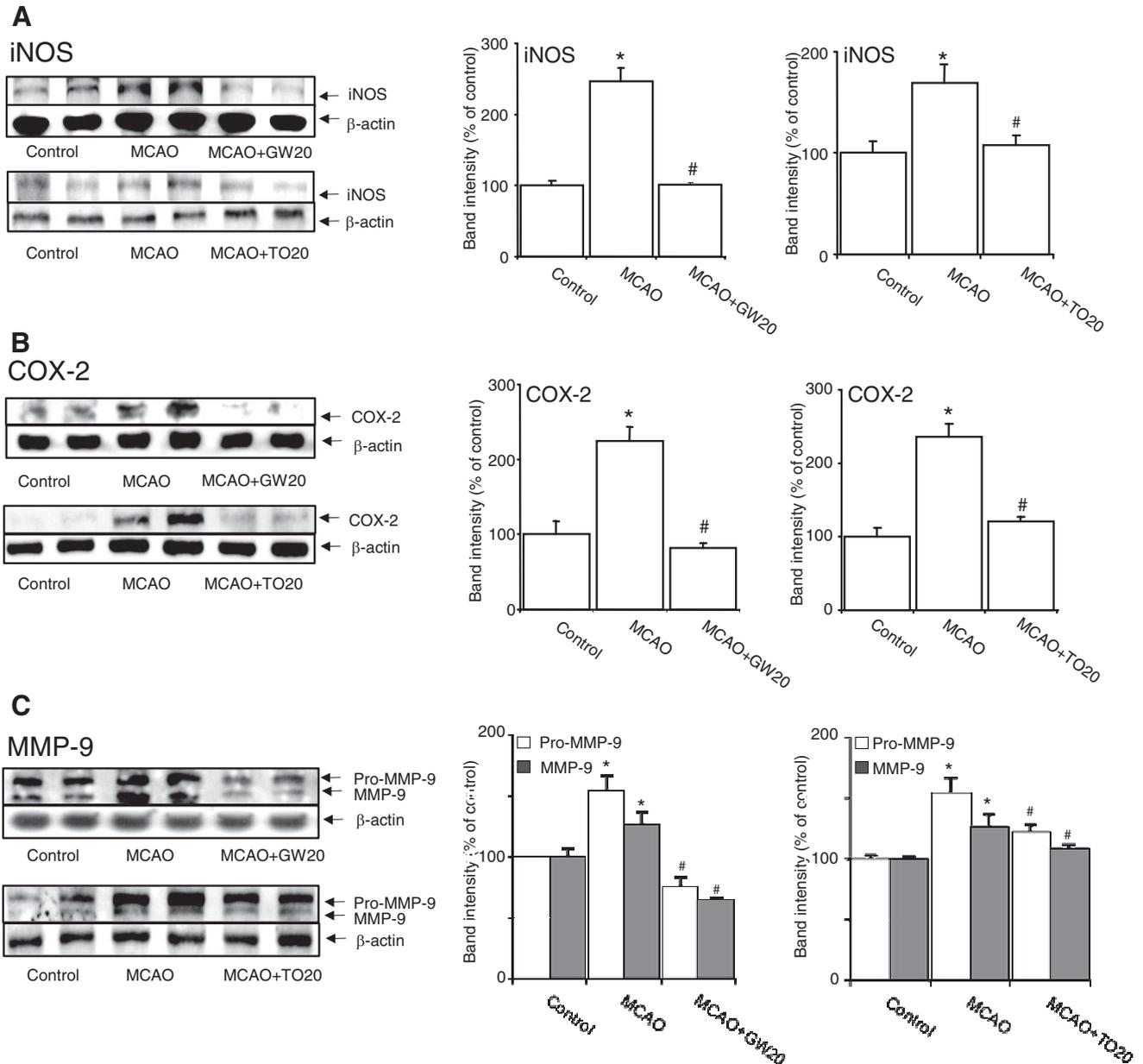
Figure 4. The LXR agonists inhibit MCAO-induced iNOS, COX-2, and MMP-9 expression after experimental stroke. Effect of GW3965 (20 mg/kg) or TO901317 (20 mg/kg) on iNOS (A), COX-2 (B), and MMP-9 (C) after permanent MCAO. A, B, and C, Western blot analyses from brain homogenates; the corresponding right panels show the densitometric analysis of bands from all blots. Data are mean±SEM; n=6; *$P<0.05$ vs control; #$P<0.05$ vs MCAO.

Infarct Outcome and Expression of Inflammatory and LXR Target Genes in LXRα,β$^{-/-}$ Mice After MCAO

Finally, we used genetic tools to analyze the effect of endogenous LXR signaling in response to stroke injury. To this end, we characterized the MCAO model in C57/BL6-Sv129 mixed-background wild-type and LXRα,β$^{-/-}$ mice. No spontaneous mortality in both mice strains after MCAO was found, and this was not affected by LXR agonists. As shown in Figure 7, administration of GW3965 agonist reduced infarct lesion in wild-type animals, whereas no significant changes were observed in LXR-deficient mice under the same experimental conditions. Of note, larger infarcted areas were observed in mice lacking both LXR isoforms

compared with control mice (Figure 7). When the neurological test was applied to this set of mice, worse performance in both neurological deficit score and grip test demonstrated a protective action in wild-type mice treated with GW3965, whereas LXRα,β$^{-/-}$ mice showed poorer neurological status (Table 2). Weight loss in wild-type mice (10.62±0.68%) was reduced by treatment with 20 mg/kg GW3965 (6.10±0.81%; n=8; $P<0.05$). However, weight loss was unaffected by treatment with LXR agonist in LXRα,β$^{-/-}$ mice (11.76± 0.56% versus 10.84±1.10% in vehicle versus 20 mg/kg GW3965; n=8; $P>0.05$).

As expected, mRNA expression of the LXR target genes ABCA1[29,30] and SREBP-1c[36] was increased by LXR agonists in wild-type mice but not in LXRα,β$^{-/-}$ mice and was
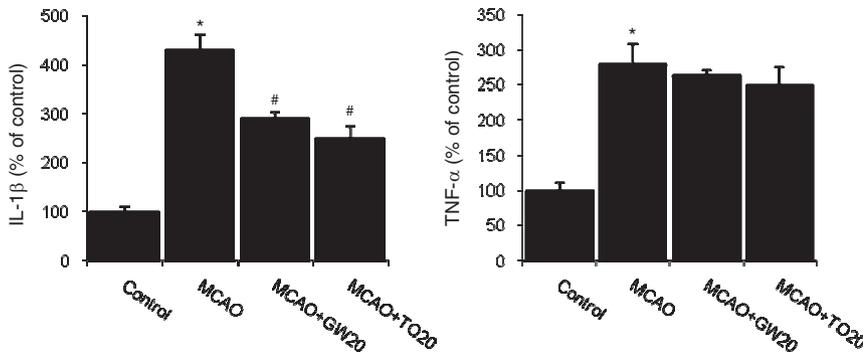
Figure 5. Effect of GW3965 (20 mg/kg) and TO901317 (20 mg/kg) on IL-1β and TNF-α levels after MCAO. IL-1β and TNF-α were determined by ELISA (see Methods for details). Data are mean±SEM; n=6; *$P<0.05$ vs control; #$P<0.05$ vs MCAO.

unaffected by the MCAO procedure. In addition, the expression of MCAO-induced NF-κB target genes such as the inflammatory cytokines IL-6 and the IL-12 p40 monomer and the chemoattractant chemokines regulated on activation, normal T cell expressed and secreted (RANTES) and monocyte chemoattractant protein-1 (reviewed elsewhere[37]) was inhibited by the LXR agonist GW3965 in wild-type but not in LXR$\alpha,\beta^{-/-}$ mice (Figure 8).

Discussion

Because the inflammatory cascade triggered by the ischemic injury in both occluded blood vessels and brain parenchyma is an important feature of the pathophysiological response to the ischemic injury, antiinflammatory strategies may be useful for acute stroke treatment. We have therefore studied the role of the LXR nuclear receptor, which is involved in cholesterol and lipid metabolism but also exerts antiinflammatory effects, on infarct outcome after experimental stroke. Our data show that the activation of the LXR receptor mediates potent neuroprotection in this setting.

First, we have explored the presence of LXR receptors both in healthy rat brain and after ischemia. Whereas LXRβ is ubiquitously expressed, LXRα expression is restricted to few tissues (reviewed in Reference 1). Here we show that LXRβ

is indeed expressed in brain at levels that are not significantly changed after exposure to ischemia, whereas the expression of LXRα is very low in brain, in agreement with previous reports showing that LXRβ is the form predominantly expressed in brain tissue.[12,13] More interesting, we have found that LXRα expression is robustly induced in rat brain after the ischemic insult. Thus, apart from LXRβ, our data support the existence of an additional target for LXR agonists in ischemic brain. This is the first evidence in the literature that LXRα is induced in brain after a deleterious stimulus such as cerebral ischemia, suggesting an endogenous role of this receptor in this pathology, as discussed below.

Because both LXR isoforms are present in brain after MCAO, we tested the effect of their activation by exogenous ligands. As LXR agonists, we have used the nonsteroidal GW3965, an LXR full agonist on both LXRα and LXRβ,[22] and the compound TO901317.[23] To elucidate whether these molecules are capable of accessing the brain, we studied their effect on a bona fide parameter of LXR transcriptional activity, the ABCA1 transporter (reviewed elsewhere[1,2]). Both caused a robust expression of ABCA1 in the ischemic brain, indicating that they cross the blood-brain barrier and exert specific actions on LXR receptors. Although previously demonstrated in mice,[38,39] our data show for the first time in
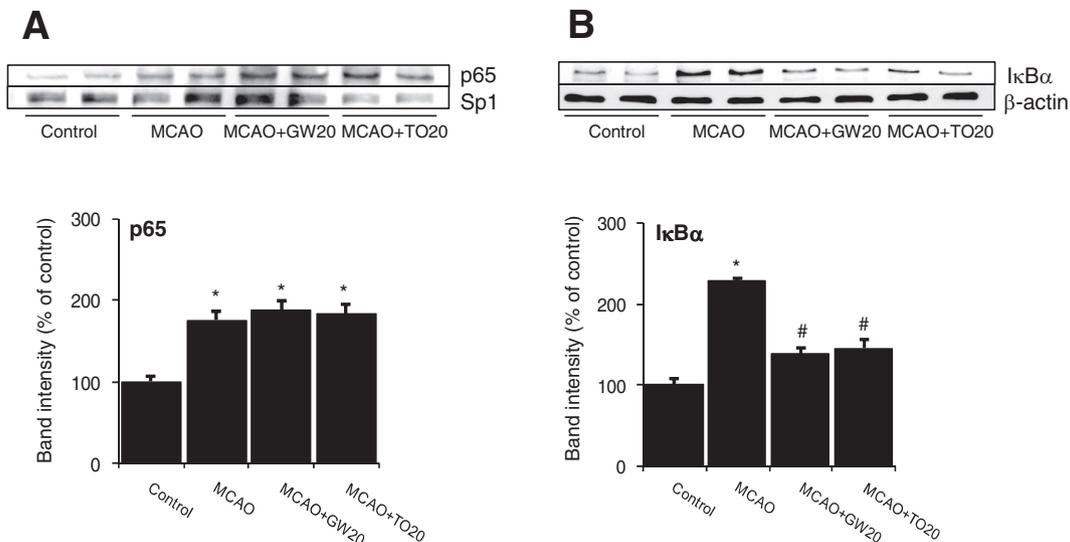
Figure 6. Effect of LXR agonists on NF-κB activation and transcriptional activity. Effect of GW3965 (GW; 20 mg/kg) and TO901317 (TO; 20 mg/kg) on nuclear p65 levels 90 minutes (A) and total IκBα protein levels 18 hours (B) after exposure to permanent MCAO determined by Western blot analysis. Bottom, Densitometric analysis of bands. Data are mean±SEM; n=6; *$P<0.05$ vs control; #$P<0.05$ vs MCAO.
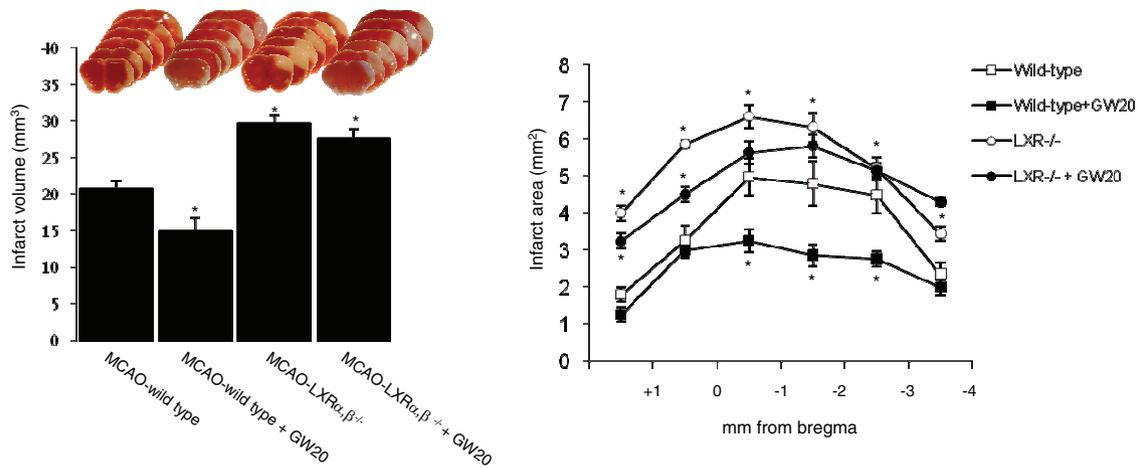
Figure 7. Infarct volumes and areas after MCAO in wild-type and LXRα,β$^{-/-}$ mice. Effect of the LXR agonist GW3965. Data are mean±SEM; n=8; *P<0.05 vs MCAO wild type (see Methods for details). Photographs are of brain slices from representative experiments.

vivo brain induction of ABCA1 after administration of LXR agonists in rats.

The LXR agonists used did not affect LXRβ expression. However, they did increase LXRα expression in control rat brain, although this effect was not apparent after MCAO, a situation with an already increased upregulation of this receptor.

More interesting, we have found that the LXR agonists are neuroprotective in experimental stroke. Indeed, both compounds, administered intraperitoneally 10 minutes or 1 hour after the ischemic occlusion, remarkably ameliorated stroke outcome, as shown by a reduction in infarct volume and in the neurological deficit induced by the ischemic injury. In the search for the mechanisms involved in this neuroprotective effect, we explored whether LXR activation inhibits ischemia-induced expression of inflammatory genes as described in macrophages exposed to bacterial pathogens.[4] Thus, we have found that both GW3965 and TO901317 inhibit MCAO-induced expression of iNOS, COX-2, and MMP-9. Whereas iNOS and COX-2 mediate cytotoxicity in many cell systems, including the ischemic brain,[31,40–44] MMP-9 is another inflammatory mediator contributing to ischemic cerebral damage[32] as a result of extracellular matrix degradation[45] and participation in hemorrhagic transformation in acute ischemic stroke in humans.[46,47] Therefore, their inhibition may explain at least partly the neuroprotective effect of these compounds. To the best of our knowledge, these results are the first evidence demonstrating that LXR agonists inhibit

COX-2 and MMP-9 expression after inflammatory stimuli in the central nervous system, which may be a useful action for different neurological disorders with an inflammatory substrate.

It has been described that the expression of iNOS, COX-2, and MMP-9 in several systems is induced by TNF-α and IL-1β. We therefore tested the effect of these agonists on the expression of these 2 cytokines induced by ischemia. Interestingly, the administration of GW3965 inhibited MCAO-induced increase in IL-1β but not in TNF-α, in agreement with previous reports on macrophage gene expression.[48]

LXR-dependent antiinflammatory properties are thought to be mediated by transrepression of factors involved in inflammatory gene expression such as NF-κB.[4,6,7] Because NF-κB is a key component in the inflammatory response after an ischemic insult in brain, we have explored whether LXR agonist–induced neuroprotection may involve disruption of NF-κB transcriptional activity, measured as expression of a bona fide NF-κB target gene, IκBα.[34,35] Indeed, we have found that administration of LXR agonists blocks MCAO-induced late increase in IκBα levels without affecting NF-κB nuclear translocation, strongly supporting that inhibition of NF-κB nuclear transcriptional activity accounts for LXR-induced neuroprotection and inhibition of brain inflammation.

To clarify whether the effects of LXR agonists were due to specific actions on LXR receptors, we have tested GW3965 on LXRα,β–genetically deficient mice. In agreement with

Table 2. Neurological Status After MCAO in Wild-Type and LXRα,β$^{-/-}$ Mice: Effect of LXR Agonists

	Wild-Type Mice		LXRα,β$^{-/-}$ Mice	
	MCAO+Vehicle	MCAO+GW3965	MCAO+Vehicle	MCAO+GW3965
Neurological deficit score	1.37±0.20	0.50±0.20*	3.12±0.24*	2.75±0.17*
Grip test (latency to fall, s)	31.6±4.1	42.8±5.7*	16.4±1.8*	15.2±1.3*

LXRα,β$^{-/-}$ mice showed lower neurological scores and poorer performance in the grip test, which was not affected by 20 mg/kg GW3965 after MCAO. This compound did improve neurological status in wild-type mice. Data are shown as mean±SEM.

*P<0.05 vs wild type MCAO+vehicle (n=8).
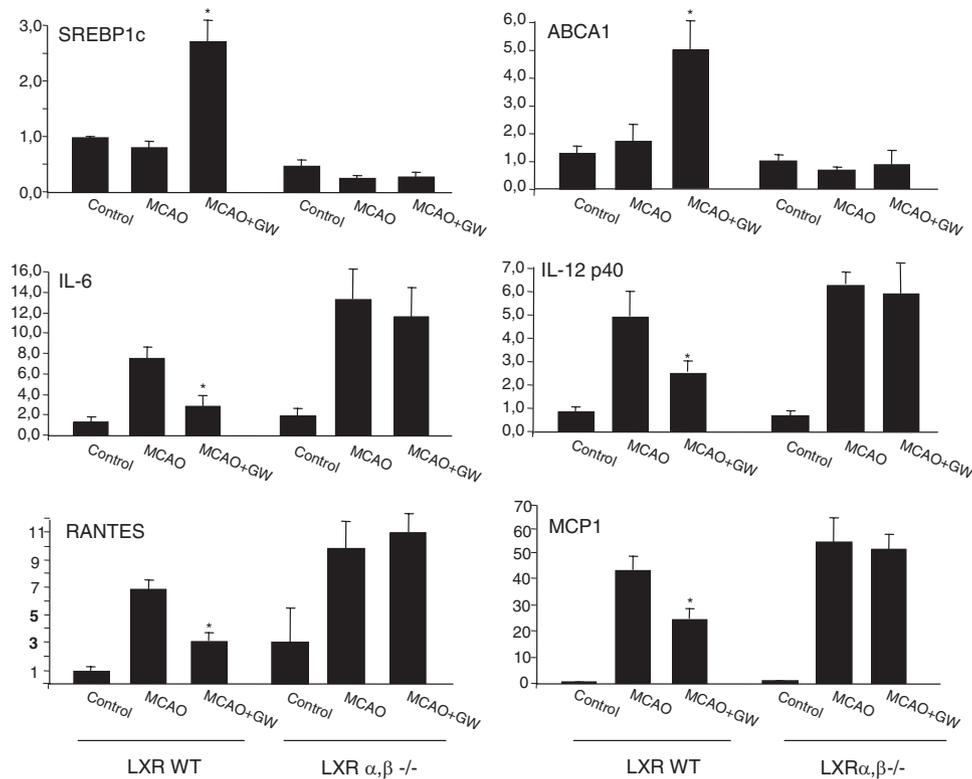
Figure 8. LXR agonists induce the expression of LXR target genes (ABCA1 and SREBP1c) and inhibit the expression of NF-κB inflammatory target genes (IL-6, IL-12 p40, regulated on activation, normal T cell expressed and secreted [RANTES], and monocyte chemoattractant protein-1 [MCP-1]) after MCAO in brain of wild-type (WT) but not of LXRα,β$^{-/-}$ mice. Expression was determined by real-time quantitative polymerase chain reaction assays. We used 36B4 as a control for RNA loading and integrity. Data are mean±SEM; n=6; *$P<$0.05 vs MCAO wild type.

our hypothesis, GW3965, on one hand, increased mRNA levels of 2 well-established LXR target genes, ABCA1 and SREBP-1c,[29,30,36] only in wild-type animals and, on the other, did not affect infarct outcome in LXRα,β$^{-/-}$ animals, confirming that the effects of GW3965 are due to activation of LXR receptors. More important, we have found that untreated LXRα,β$^{-/-}$ mice had a much greater infarct volume than wild-type mice. Furthermore, MCAO-induced mRNA expression of several inflammation-related, NF-κB target genes was abolished in wild-type but not in LXRα,β$^{-/-}$ mice, thus confirming that LXR activation induces neuroprotection in experimental stroke by inhibiting NF-κB–induced transcriptional activity and inflammation. These data show for the first time that an endogenous LXR activator pathway during experimental stroke mediates a potent and natural protection, which could be caused by physiological LXR agonists such as oxysterols.[49] This piece of evidence suggests that levels of endogenous LXR agonists might serve as prognostic markers in stroke patients; moreover, alterations in the expression or function of LXR receptors resulting from polymorphisms or other causes may severely affect stroke outcome and conceivably other pathologies in which LXR nuclear receptors are involved such as atherosclerosis.

Previous evidence has demonstrated that the antiinflammatory action of LXR synthetic agonists can be mediated through either LXRα or LXRβ.[4,5] Moreover, recent work from Zelcer and colleagues[50] demonstrated that loss of either LXRα or LXRβ expression exacerbates Alzheimer's disease–related pathology in APP/PS1 transgenic mice. Although unexplored in LXRα$^{-/-}$, data exist showing that loss of LXRβ is associated with some central nervous system pathologies.[51,52] Therefore, further studies are needed to clarify the role of each LXR isoform in the cerebral ischemia scenario.

Conclusions
Our results demonstrate that the activation of the LXR receptor exerts potent neuroprotective actions in experimental stroke as a result of the inhibition of inflammatory mediators. Given that these compounds were administered after the onset of the ischemic damage, our findings may possess therapeutic repercussions in the management of acute ischemic stroke.

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None.

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CLINICAL PERSPECTIVE

Liver X receptors (LXRs) α and β are ligand-activated transcription factors that belong to the nuclear receptor superfamily. LXRs regulate the expression of a number of genes involved in cholesterol metabolism. In addition, LXRs are known to antagonize the expression of a panel of inflammatory genes. All these pieces of evidence suggest that LXR activation may exert a protective role in pathologies in which inflammation is involved such as stroke. Taking into account the epidemiological importance of stroke and the limited possibilities for treatment, activation of LXR might arise as a possible powerful approach for stroke treatment. The present results demonstrate that the activation of the LXR receptors exerts potent neuroprotective actions in experimental stroke, which are concomitant to the inhibition of inflammatory mediators. Apart from the possible therapeutic repercussions in acute stroke management, these findings suggest, on one hand, that the endogenous levels of LXR agonists such as oxysterols could serve as a helpful prognostic marker in stroke patients and, on the other, that polymorphisms or other alterations of the LXR receptor expression or function may increase vulnerability to stroke.