# A New Target for Stroke

Eyal Leibovitz, MD, McGill University, Montreal, Quebec, Canada, gave a brief tutorial on PPARs (peroxisome proliferator-activated receptors) and presented an overview of the current knowledge concerning the effects of PPAR agonists on the vascular bed.

Peroxisomes are intracellular organelles that are predominant in nearly all mammalian cells. PPARs received their name when it was shown that the activation of these receptors was associated with proliferation of peroxisomes in rodents. There are 3 types of PPARs: PPARα, PPARγ, and PPARβ. The natural activators of the PPARs are fatty acids, but some prostaglandins and leukotrienes also activate them, and they can also be activated by synthetic compounds, including fibrates, which activate PPARα and are an accepted treatment for dyslipidemia, and the thiazolidinediones (TZDs), which activate PPARγ and are an accepted treatment for type 2 diabetes mellitus. PPARβ/δ activators are experimental and are not used clinically.

Both PPARα and γ are expressed in human vascular endothelial cells [Delerive P et al. *Circ Res* 1999], and animal studies have shown that PPARα and γ may exert vascular protective effects in hypertension and other forms of cardiovascular disease by interfering with signaling pathways that lead to endothelial dysfunction, vascular remodeling, inflammation, oxidative stress, and the growth and progression of atherosclerosis [Diep et al. *Circulation* 2002; Diep et al. *Hypertension* 2002; Collins AR et al. *ATVB* 2001].

Clinical evidence regarding the vascular effects of PPAR activators come from studies of dyslipidemia (PPARα activators) and diabetes mellitus (PPARγ activators). The use of fibrates has been clearly shown to reduce morbidity and mortality among diabetic dyslipidemic patients; however, the results among nondiabetic dyslipidemic individuals, especially those with low HDL levels, are not as clear. The results with PPARγ activators, although effective in controlling metabolic aspects of diabetes, remain controversial from the point of view of cardiovascular protection.

Although the use of selective PPARγ activators may exert vascular protective effects in hypertension or other forms of cardiovascular disease, Dr. Leibovitz concluded with a caution that one of the PPARγ agonists has been associated with a significant increase in the risk of myocardial infarction and heart failure, and with an important but not statistically significant increase in the risk of death from cardiovascular causes [Nissen SE, Wolski K. *NEJM* 2007; Nissen SE et al. *JAMA* 2005].

Frank M. Faraci, PhD, University of Iowa, Iowa City, IA, and his colleagues have been studying mice that have been genetically altered to express dominant-negative mutations of the human PPARγ gene (PPARγ P465L, for example). In these "humanized" mice the subsequent interference with PPARγ signaling caused selective endothelial dysfunction in both cerebral arteries and arterioles. The impact of PPARγ interference was prominent in the cerebral circulation and the aorta was relatively normal in these mice.

Levels of superoxide (an oxygen-derived free radical) were increased in cerebral arterioles, and impaired endothelial function could be restored to normal with a scavenger of superoxide in PPARγ P465L mice, suggesting that mechanisms that account for impairment of cerebral vascular function following interference with PPARγ involve oxidative stress.

The implications of these findings extend far beyond regulation of vascular tone, because the endothelium affects vascular structure, blood cells, and neuronal function. Other studies

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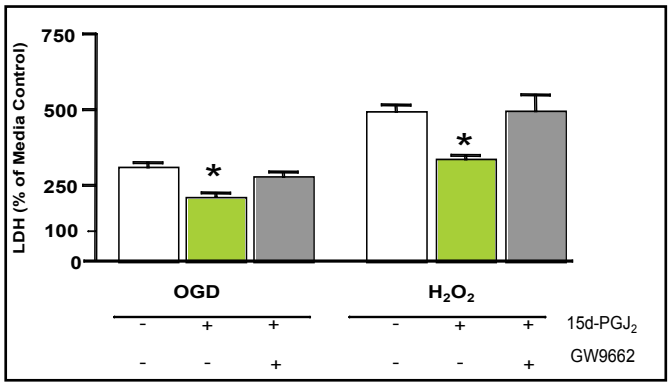
that have been conducted by Dr. Faraci and colleagues have shown that interference with PPARγ, either systemically or specifically in vascular muscle, produces vascular hypertrophy and inward growth of cerebral arterioles. These findings indicate that PPARγ normally inhibits vascular growth and inward vascular remodelling—effects that have a significant impact on local hemodynamics.

Future research will focus on identifying the mechanisms that promote oxidative stress and alter vascular growth, further defining the cell-specific role for PPARγ, and determining whether overexpression of wild-type PPARγ protects against vascular disease.

Jaroslaw Aronowski, PhD, University of Texas, Houston, TX, reviewed the results of several animal studies that focused on the neuroprotective, cytoprotective, and neuroinflammation role of PPARγ following an intracerebral hemorrhage (ICH).

Employing an excitotoxic, ischemia-like (oxygen-glucose-deprivation; OGD), or oxidative stress (hydrogen peroxide; H2O2) injury to neurons, Aronowski and colleagues have shown that PPARγ significantly reduces neuronal death (Figure 1). This neuroprotective effect was linked to increased PPARγ DNA-binding activity [Brain Res. 2006].

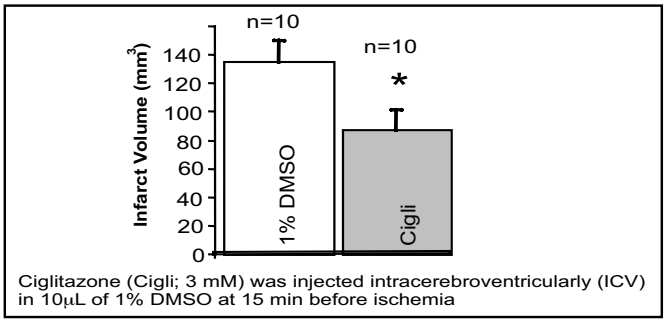
**Figure 1. PPARγ Activator Reduces ODG- and $H_2O_2$-Mediated Damage.**




In another study, injection of 15d-Delta(12,14)-prostaglandin J(2) (15d-PGJ(2)), a proposed endogenous PPARγ agonist, into the locus of striatal hematoma increased PPARγ DNA-binding activity, the expression of catalase messenger ribonucleic acid (mRNA), and protein in the perihemorrhagic area. Additionally, 15d-PGJ(2) significantly reduced nuclear factor-kappaB (NFκB) activation and prevented neutrophil infiltration, reduced neuronal death, and reduced behavioral dysfunction produced by the ICH [Zhao X et al. *J Cereb Blood Flow Metab* 2006].

The PPARγ agonist rosiglitazone stimulated primary microglia in culture toward phagocytosis of red blood cells. Rosiglitazone also promoted hematoma resolution, decreased neuronal damage, and improved functional recovery in a mouse ICH model. PPARγ activators significantly increase PPARγ-regulated gene expression (catalase and CD36) and reduce proinflammatory gene expression [Zhao X et al. Ann Neurol 2007]. Intraventricular injection of ciglitazone or 15d-PGJ(2) into ischemic rat brains significantly increased the PPARγ DNA-binding activity and reduced infarction volume at 24h after reperfusion [Figure 2; Ou Z et al. Brain Res 2006].

**Figure 2. Intraventricular Injection of Ciglitazone Reduced Infarct Volume 24h After Reperfusion.**




Ciglitazone (Cigli; 3 mM) was injected intracerebroventricularly (ICV) in 10μL of 1% DMSO at 15 min before ischemia

All of these results suggest that PPARγ may be beneficial in protecting brain cells from ICH-induced damage. These positive results in animal stroke models have encouraged the experimental study of PPARγ in patients.

Maria A. Moro, PhD, Universidad Complutense, Madrid, Spain, reviewed the results of several animal studies that focused on the neuroprotective and anti-inflammatory role of PPARγ agonists in experimental stroke models and discussed some recent clinical results involving PPARγ ligands that have been shown to improve outcome following ischemic stroke.

Using rats that were exposed to middle cerebral artery occlusion, administration of the PPARγ agonists rosiglitazone, 15d PGJ(2), or L-796,449 after the ischemic onset decreased infarct volume and neuroinflammation, as well as NFκB transcriptional activity [Pereira et al. *J Exp Neurol* 2005; Pereira et al. *J Cereb Blood Flow Metab* 2006].

In addition, neuroprotective actions of PPARγ have been shown to go beyond inflammation. A recent study has shown that GLT1/EAAT2, the major glutamate transporter in the central nervous system, is a PPARγ target gene. Upregulation of the expression of this transporter caused by PPARγ activation decreases excitotoxicity and subsequent neuronal death [Romera et al. *J Cereb Blood Flow Metab* 2007].

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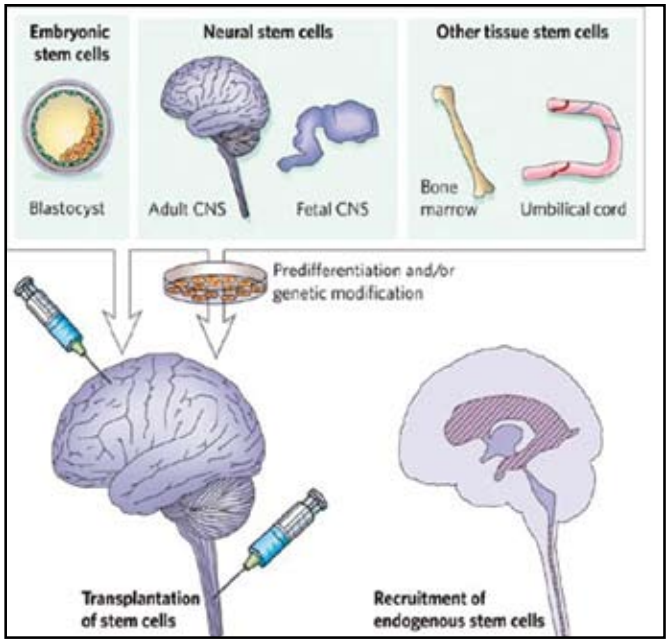
(Hummel FC, Cohen LG. *Lancet Neurology* 2006). The types of issues that need resolution include optimization of the stimulation site, technique optimization, characterization of the patients/injuries/tasks that may be helped, and the safety of the procedures (Tallelli P, Rothwell J. *Curr Opin Neurol* 2006; Fregni F, Pascual-Leone A. *Cogn Behav Neurol* 2006).

Cellular therapies are also being explored as a mechanism for brain repair after stroke, as discussed in an overview given by Sean Savitz, MD, University of Texas, Houston, TX. The concept arose from stem cell transplantation in cancer patients as well as transplantation in those with Parkinson disease. Promotion of lost neuronal connections and conductivity, enhancement of trophic support for neurogenesis, angiogenesis, synaptogenesis, prevention of cell death, and reduction of inflammatory responses and scar formation are some of the possible mechanisms whereby cell therapy could enhance brain recovery. Although it is an exciting idea, it is an area that is full of challenges. "Is it really possible to consider that cellular therapy or cellular transplantation is going to reconstruct the complex tapestry of the infarcted brain?" asked Dr. Savitz. Some of the parameters that researchers must determine are the infarct size and location, the timing of therapy, injection sites, routes of delivery, which cell types (Figure 1), and patient safety monitoring. The search for an effective therapy to promote brain repair after stroke continues to evolve across the domains of physical therapy, brain stimulation, and cell therapy.

**Figure 1. Complexity of Cell Types.**

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In a large group of stroke patients who were admitted within 24 hours of symptom onset, plasma levels of 15dPGJ(2) on admission were significantly higher than in control patients. A linear relationship between increased plasma 15-dPGJ(2) concentration and better neurological outcome at 3 months, less neurological deterioration, and smaller infarct volume was noted, indicating a neuroprotective effect for 15-dPGJ(2) in atherothrombotic ischemic stroke [Blanco M et al. *Stroke* 2005].

In another clinical study, the use of PPARγ was associated with enhanced functional recovery in stroke patients with type 2 diabetes compared with a control group [Lee J, Reding M. *Neurochem Res* 2007].

Dr. Moro feels that experimental evidence together with these early clinical results shows a need for larger clinical studies that use PPARγ agonists as potential therapeutic agents not only for prevention but also for treatment of acute stroke.

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Seven subjects were successfully implanted with 5-7 microstimulators. After a 12-week period of functional exercise using personalized activity programs supported by electrical stimulation, improvement was noted in function (ARAT scores), impairment motor scores (Fugl-Meyer), motor control (Tracking Index), and spasticity (Stretch Index). The largest gains were seen in patients <2 years post-stroke. There were no infections or delayed wound healing. Six of the seven subjects continue to use the system at home. Dr. Burridge looks forward to the next generation of microstimulators and the feasibility of using fewer devices.