Activation of Peroxisome Proliferator–activated Receptor β/δ Attenuates Acute Ischemic Stroke on Middle Cerebral Ischemia Occlusion in Rats

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Background: Peroxisome proliferator–activated receptor (PPAR)-β/δ is a transcription factor that belongs to the nuclear hormone receptor family. There is little information about the effects of the immediate administration of specific ligands of PPAR-β/δ (GW0742) in animal models of acute ischemic stroke. Using a rat model of middle cerebral ischemia occlusion (MCAO) in vivo, we have investigated the effect of pretreatment with GW0742 before MCAO. *Methods:* The neuroprotective effect of GW0742 against acute ischemic stroke was evaluated by the neurologic deficit score (NDS), dry–wet weight, and 2,3,5-triphenyltetrazolium chloride staining. The levels of interleukin (IL)-1β, nuclear factor (NF)-κB, and tumor necrosis factor (TNF)-α were detected by an enzyme-linked immunosorbent assay. The expressions of inducible nitric oxide synthase (iNOS), Bax, and Bcl-2 were detected by Western blot. The apoptotic cells were counted by in situ terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay. *Results:* The pretreatment with GW0742 significantly increased the expression of Bcl-2, and significantly decreased in the volume of infarction, NDS, edema, expressions of IL-1β, NF-κB, TNFα, and Bax, contents of iNOS and the apoptotic cells in infarct cerebral hemisphere compared with rats in the vehicle group at 24 hours after MCAO. *Conclusions:* The study suggests the neuroprotective effect of the PPAR-β/δ ligand GW0742 in acute ischemic stroke by a mechanism that may involve its anti-inflammatory and antiapoptotic action. **Key Words:** PPAR-β/δ—GW0742—middle cerebral artery occlusion (MCAO)—anti-inflammatory—rat.

Introduction

Stroke is the second leading cause of death in industrialized countries and the most important cause of acquired adult disability.[1] Nearly one third of patients with acute ischemic stroke develop early neurologic deterioration, a situation associated with increased mortality and long-term functional disability.[2] The mechanisms underlying neural cell injury in cerebral ischemic are not yet

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well understood, but many studies have clearly shown that inflammatory response and oxidative stress have been found to play an important role in the pathogenesis of cerebral ischemia.[3]

Peroxisome proliferator–activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily.[4] There are three members of the PPAR subfamily: PPAR-α, PPAR-β/δ, and PPAR-γ. PPARs have been implicated in the pathogenesis of a number of diseases, including diabetes mellitus, obesity, atherosclerosis, and neurologic diseases.[5] There is good evidence that WY14643, a selective PPAR-α agonist, and rosiglitazone and pioglitazone, two PPAR-γ agonists, have the neuroprotective effect on cerebral ischemia.[6,7] However, there is less information about the role of ligands of PPAR-β/δ in acute ischemic stroke.

PPAR-β/δ exerts a strong protection from renal and gut ischemia–reperfusion (I/R) injury.[8,9] And many studies had clearly shown that GW0742, a potent PPAR-β/δ agonist, exerts anti-inflammatory effects in atherosclerosis, myocardial infarction, endotoxic shock, and lung inflammation.[10-13] Recent research indicated that PPAR-β/δ plays an important role in integrating and regulating central inflammation and food intake after middle cerebral ischemia occlusion (MCAO).[14]

So the aim of our present study was to examine the neuroprotective effect of the PPAR-β/δ ligand GW0742 on MCAO in rats and its potential mechanisms.

Materials and Methods

Animals

Adult male Sprague–Dawley rats weighing 260-280 g and aged 11-12 weeks were purchased from Xi'an Jiaotong University Animal Services for this study. The protocol was approved by the institutional animal care and use committee and the local experimental ethics committee. All rats were allowed free access to food and water before surgery under optimal conditions (12/12 hours light/dark with humidity of 60 ± 5% and temperature of 22 ± 3°C).

Model of MCAO

MCA thread occlusion was induced according to the previously published methods.[15] Rats were anesthetized with chloral hydrate at the dose of 380 mg/kg, intraperitoneally. The right common carotid artery was exposed and separated carefully from the vagus nerve and ligated at the more proximal side through a right paramedian incision. The external carotid artery was ligated. The occipital and pterygopalatine arteries were coagulated. Ischemia was produced by advancing a tip rounded .30-mm nylon suture into the internal carotid artery through the external carotid artery. Reperfusion was produced by withdrawal of the intraluminal nylon suture. The physiological variables in rats were measured at baseline, during MCAO and at reperfusion. The right femoral artery was cannulated for continuous monitoring of blood pressure and arterial blood sampling. A rectal probe was inserted to monitor core temperature. Arterial blood gases and plasma glucose were measured at baseline, during MCAO and at reperfusion.

Experimental Protocol

Male Sprague–Dawley rats were randomly divided into 3 groups: sham group; vehicle group, which was pretreated with saline at 30 minutes before MCAO; GW0742 group, which was pretreated with GW0742 .3 mg/kg (intraperitoneally) at 30 minutes before MCAO. The rats in each of the groups were subdivided into 5 subgroups consisting of 8 animals. The first subgroup was used for neurologic deficit score (NDS) and 2,3,5-triphenyltetrazolium chloride staining; the second subgroup was used for brain water content; the third subgroup was used for detecting nuclear factor (NF)-κB, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β by an enzyme-linked immunosorbent assay; the fourth subgroup was used for detecting inducible nitric oxide synthase (iNOS), Bcl-2, and Bax by Western Blot; and the fifth subgroup was used for terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL). The sham group was only subjected to surgical procedures, whereas other animals were subjected to focal ischemia by MCAO using intraluminal thread, and after 2 hours of MCAO reperfusion allowed by retracting the thread.

Evaluation of Neurologic Impairment

Twenty-four hours after MCAO, the neurologic deficit of each rat was evaluated according to the Longa method of a 5-point scale[16] by the same experimenter, who was blinded to the different treatments in the experiment (no neurologic deficit = 0, failure to extend right paw fully = 1, circling to right = 2, falling to right = 3, and being unable to walk spontaneously and depression of consciousness = 4).

Measurement of Infarct Volume

After the NDS, rats were killed and their brains quickly removed and frozen at −20°C for 15 minutes. Coronal brain sections (2-mm thick) were stained with 2% 2,3,5-triphenyltetrazolium chloride at 37°C for 20 minutes followed by fixation with 4% paraformaldehyde. Brain slices were scanned individually and the unstained area was analyzed by the image analyzing system (Adobe Systems Incorporated, San Jose, CA). To compensate for the effect of brain edema, the corrected volume was calculated using the following equation: percentage hemisphere lesion volume (% HLV) = {[total infarct volume − (right hemisphere volume − left hemisphere volume)]/left