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Telmisartan mediates anti-inflammatory and not cognitive function through PPAR-γ agonism via SARM and MyD88 signaling

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ABSTRACT

Telmisartan (TM), an angiotensin II receptor I (AT1) blocker, has been reported to have agonist property with respect to PPAR-γ. Activation of PPAR-γ receptor by TM attenuated the lipopolysaccharide (LPS) mediated TLR4 central downstream inflammatory responses. However, the missing link between PPAR-γ and TLR4 signaling with TM stimulation has not been clarified. Hence, the present study has been designed to evaluate the molecular mechanism involving PPARγ–TLR4 signaling with TM stimulation in LPS induced inflammatory model. LPS was administered in rats through ICV and the rats were treated with either PPAR-γ antagonist GW9662 (GW) or TM or both. After 14 days of LPS administration, the rats were subjected to behavioral tests and their brains were isolated for blotting techniques. The protein study includes NF-κB, PPAR-γ receptors, and their downstream proteins (MyD88 & SARM). The pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) levels were measured by ELISA and cresyl violet staining in the hippocampus region to measure the neuroprotective activity. Results have shown that TM significantly increased the motor co-ordination, cognitive functions, and activated SARM and PPAR-γ protein levels. Also, TM treatment decreased the NF-κB, MyD88 activation, and cytokines release in LPS rats. The co-administration of GW attenuated the TM responses in the parameters studied except cognitive functions. TM (10 mg/kg) has significantly reduced the LPS mediated inflammatory responses. This resulted in effective regeneration of hippocampal neurons as observed by cresyl violet staining. It can be concluded that the activation of PPAR-γ receptors may increase the SARM and decrease the MyD88 and NF-κB expression. This negative regulation of SARM dependent inflammation control could be a possible mechanism for TM anti-neuroinflammatory activity. This study of TM in neuro-inflammatory model may further confirm the dual activities of TM that controls hypertension and cognition through AT1 blockade and also attenuates neuro-inflammation via PPAR-γ agonistic property.

1. Introduction

Neuroinflammation (NI) refers to inflammation in the nervous tissues. The factors contributing inflammation include bacterial or viral infections, neuronal injury, chronic inflammatory syndromes, and induction through environmental toxins (Block and Hong, 2005). During NI, the permeability of the blood brain barrier (BBB) will increase to allow the infiltration of lymphocytes, macrophages, and perhaps toxins into the brain parenchyma (Rivest, 2010). NI may turn on the host defense mechanism in the nervous tissues through activation of microglia and astrocytes, leading to the release of inflammatory cytokines and oxy radicals (Minghetti et al., 2005; Moynagh, 2005). NI is closely associated with several neurodegenerative disorders including multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, and cerebral ischemia (Gonzalez et al., 2014). Several experimental findings have shown control of inflammatory responses with telmisartan (TM) treatment, an angiotensin II receptor 1 (AT_1) blocker. The TM effect was attributed to its BBB permeation and anti-inflammatory properties (Noda et al., 2012). Treatment with TM (5 mg/kg) suppressed the cerebral injury in a murine model of transient focal ischemia and the effect was correlated with anti-inflammatory and antioxidant properties (Kasahara et al., 2010). Reports have shown that TM ameliorated the neuronal inflammatory response by blocking c-Jun N-terminal kinases (JNK/c-Jun) and nicotinamide adenine dinucleotide phosphate (NADPH)

Abbreviations: AP-1, activator protein-1; ANOVA, analysis of variance; AT_1, angiotensin II receptor 1; BBB, blood brain barrier; C, control; $CaCl_2$, calcium chloride; CMC, carboxy methyl cellulose; CSF, cerebrospinal fluid; JNK/c-Jun, c-Jun N-terminal kinases; COX-2, cyclooxygenase-2; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; EGTA, ethylene glycol tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; GW, GW9662; i.v, intravenous; iNOS, inducible nitric oxide synthase; IRFs, interferon regulatory factors; IFNs, interferons; ICV, intracerebroventricular; LPS, lipopolysaccharide; $MgCl_2$, magnesium chloride; MCA, middle cerebral artery; MAP, mitogen activated protein; MCP-1, monocyte chemotactic protein-1; MyD88, myeloid differentiation factor 88; NI, neuroinflammation; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; PPAR-γ, peroxisome proliferator-activated receptor-γ; PMSF, phenylmethylsulfonyl fluoride; KCl, potassium chloride; SO, sham operated; NaCl, sodium chloride; SDS, sodium dodecyl sulfate; SD, Sprague Dawley; SEM, standard error of mean; SARM, sterile alpha and HEAT/Armadillo motif; TM, telmisartan; TLR, toll like receptors; TRIF, toll-interleukin 1 receptor domain-containing adaptor-inducing interferons; TNF-α, tumor necrosis factor-α.

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oxidase pathways in neuronal culture (Pang et al., 2012). Further, TM attenuated the monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) levels in the cerebral cortex of middle cerebral artery (MCA) occluded spontaneously hypertensive stroke-resistant rats, which confirms the anti-inflammatory activity of TM (Sato et al., 2014). Though many studies state that the anti-inflammatory property of TM is an AT_1 receptor mediated response, the concurrent peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist activity of TM cannot be ignored. Reports have supported that PPAR-γ activation of TM also contributes to the attenuation of NI (Garrido-Gil et al., 2012; Iwanami et al., 2010), but the molecular mechanism behind PPAR-γ dependent TM neuroprotective activity remains to be elucidated.

Lipopolysaccharide (LPS) is a bacterial endotoxin and exhibits central inflammatory responses through member of toll like receptors (TLR) like TLR4, which is widely expressed in glial cells, particularly in microglia and astrocytes (Kielian, 2006). TM dose-dependently attenuates the LPS mediated release of pro-inflammatory mediators, mitogen activated protein (MAP) kinases, and activation of nuclear factor-κB (NF-κB) inflammatory pathways in circulating human monocytes through agonistic activity at PPAR-γ (Pang et al., 2011). Elucidating the PPAR-γ agonistic property and cell signaling pathways of TM in NI animal model can clarify the link between PPAR-γ activation and regulation of signal transduction proteins. This study also aimed to know the missing link between PPAR-γ and TLR4 signaling. Therefore, the present study was designed to investigate the PPAR-γ agonistic property of TM in LPS mediated neuroinflammatory events in Sprague Dawley (SD) rats. TM neuroprotective effect was evaluated upon intracerebroventricular (ICV) administration of LPS which is an optimum model to mimic the NI condition (Kovacs et al., 2011). Further the alteration of signal transduction proteins (myeloid differentiation factor 88-MyD88, NF-κB & sterile alpha, and HEAT/Armadillo motif-SARM) and PPAR-γ were studied using blotting techniques to understand the molecular mechanism and the role of PPAR-γ in TM induced neuroprotection. The behavioral studies and cresyl violet staining in hippocampus region were carried out to support the neuroprotective activity of TM in NI model.

2. Materials and methods

2.1. Chemicals and reagents

TM was gift sample from Zydus Cadila, Ahmedabad, India. LPS (*Escherichia coli*, serotype 055:B5), Ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, pepstatin, and GW9662 were purchased from Sigma, St. Louis, USA. ECL plus Western detection kit and hydrophobic polyvinylidene difluoride membranes were procured from Amersham Pharmacia Biotech, Piscataway, New Jersey, USA. Antibodies of anti-MyD88, anti- phospho-PPARγ, anti-phospho-p65 (NF-κβ), and anti-SARM were purchased from Santa Cruz Biotech Inc, Dallas, Texas, USA. All reagents were prepared using deionized (Millipore)/glass-distilled water.

2.2. Animals

Male Sprague Dawley (SD) rats (each weighing 170–200 g and 6 months old) were used in this study. Rats were supplied from central animal house facilities, PSG Institute of Medical Sciences and Research (Coimbatore, India). Animals were housed in polypropylene cages and maintained under standard housing condition (room temperature 25 ± 2 °C and relative humidity 55%) with 12 h light and 12 h dark cycle. Animals had free access to food and purified water ad libitum. All the experimental animals were accommodated at least for 7 days to adapt to the laboratory conditions before starting the experiments. All the animal experimental procedures were carried out according to the "Guide for Care and Use of Experimental Animals in Research" (Indian Council of Medical research, 1992). The experimental protocol on animal subjects or that procedure is in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). The Institutional Animal Ethical Committee approved the study protocol (proposal authorization number-158/99/CPCSEA/171).

2.3. Experimental design and drug treatment

The rats were divided into seven groups; each group consists of nine rats. The first three groups were control and were sham operated (only for behavior studies), while the LPS groups received 0.3% carboxy methyl cellulose (CMC) orally as vehicle treatment. The fourth, fifth, and sixth test group animals were treated with PPAR-γ antagonist GW9662 4 mg/kg (GW), AT_1 blocker telmisartan 5 mg/kg (TM5) and telmisartan 10 mg/kg (TM10) respectively. Finally, the seventh group of rats received both GW and TM10. All the remaining groups except the control and sham operated (SO) groups were administered with LPS 3 μg through ICV route. TM10 (oral) and GW (intravenous) were treated 2 weeks and 30 min prior to LPS administration respectively.

2.4. Surgical procedure

Rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). After anesthesia, ICV administration of LPS was made by using stereotaxic apparatus as per following procedure (Gartlon et al., 2001). The rat head was positioned in a stereotaxic frame and a midline sagittal incision was made in the scalp. A Burr hole was drilled over the fourth ventricle in the skull using the following coordinates: 2.5 mm posterior from lambda, on the midline, 7 mm below the dura. ICV injection of LPS (3 μg/10 μl), dissolved in artificial Cerebrospinal fluid (CSF) was slowly injected using a Hamilton micro-syringe. In the SO group, artificial CSF (147 mM NaCl; 2.9 mM KCl; 1.6 mM $MgCl_2$; 1.7 mM $CaCl_2$ and 2.2 mM dextrose) alone was injected in the same volume. After surgery each rat was given a subcutaneous injection of 0.3 mg buprenorphine hydrochloride and ringer lactate 1.5 ml. Post-operative care included neomycin topical ointment applied in the exposed skull and scalp prior to closure. Lidocaine was applied locally to the scalp to reduce pain, and 5 ml of sterile isotonic saline-injected subcutaneously to prevent dehydration during recovery. The rats were closely monitored until recovery. Body weight and temperature were monitored periodically. After a recovery period of 14 days the rats were tested for the behavior and learning parameters using an actophotometer and a morris water maze.

2.5. Behavioral assessments

2.5.1. Morris water maze

The water maze is a circular pool with a diameter of 120 cm and a height of 50 cm and is divided into four quadrants filled with water and maintained at a temperature of 24 ± 1 °C. Initially, a visible platform test was performed, which will confirm that there will be no significant differences in sensory, motor, or motivational activities between the groups. Consequently, hidden platform and reverse hidden platform tests were conducted in succession. For the hidden platform test, a round platform with a diameter of 9 cm was placed at the midpoint of the fourth quadrant, 2 cm below the water surface. A training trial was conducted once a day for 5 days. During each trial, the rats were placed in the water at a fixed position, opposite the platform and at the edge of the pool. The rats were allowed to swim freely until they escaped onto the platform. Swimming time, escape latency time, swim