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Non-hypotensive dose of telmisartan and nimodipine produced synergistic neuroprotective effect in cerebral ischemic model by attenuating brain cytokine levels

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ABSTRACT

The hypothesis of the present study is that the anti-inflammatory property of telmisartan (TM), an AT_1 blocker that may exert neuroprotection through attenuation of excitatory amino acids by controlling cytokines and reactive oxygen species, release during ischemia. The neuroprotective effect of TM and its combination with nimodipine (NM) were studied in rats by using middle cerebral artery occlusion method followed by ischemic reperfusion (IR) after 2 h of occlusion. The drugs were administered 30 min prior to the surgery and continued throughout the study period. After 24 h of IR the neurological deficit was assessed, and the locomotor activity and open field behaviour were assessed on the seventh day. On the ninth day, the brains were isolated for neurochemical and cytokine measurements and histopathological studies. The results have shown that treatment of TM (5 & 10 mg/kg) gradually reduced the glutamate, aspartate and glutamine synthetase levels. It also restored the ATP, Na^+K^+ATPase, glutathione and synapse integrity in the different regions of the brain in comparison to ischemic brain. TM ameliorated the pro-inflammatory cytokine (IL-1β, IL-6, TNF-α), lipid peroxide and nitric oxide levels. Anti-inflammatory cytokine IL-10 level was found to be concurrently increased. Combination therapy of TM with NM (5 mg/kg) has shown additive effects in the above said parameters. Further a positive correlation between glutamate and cytokine release was observed, and it indicated that synaptic clearance of glutamate can be regulated by cytokines. It can be concluded that TM induces neuroprotective activity through amelioration of pro-inflammatory cytokine release during cerebral ischemia. The additive effect of NM on TM neuroprotective effect would be through controlling cytokine release, ATP restoration by cerebrovasodilation, and along with prevention of Ca^{2+} dependent glutamate toxicity in neurons. The advantage of TM therapy in ischemic state can be explored clinically due to its dual effect in hypertension.

Abbreviations: [Ca^{2+}], calcium ion; [K^+], potassium ion; [Na^+], sodium ion; ANOVA, analysis of variance; ANSA, 1-amino-2-napthol-4-sulphonic acid; AT_1, angiotensin receptor 1; ATP, adenosine triphosphate; BBB, blood brain barrier; BHT, butylated hydroxyl toluene; BSA, bovine serum albumin; CMC, carboxy methyl cellulose; CNS, central nervous system; DNA, deoxyribonucleic acid; DNPH, dinitrophenyl hydrazine; DTNB, dithiodinitrobenzene; EAAs, excitatory amino acids; EDTA, ethylenediaminetetraaceticacid; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; GLT-1, glutamate transporter-1; GS, glutamine synthetase; GSH, reduced glutathione; HCl, hydrochloric acid; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; i.p, intra-peritoneal; IL, interleukin; iNOS, inducible nitric oxide synthase; IR, ischemic reperfused; JNK/c-Jun, c-Jun N-terminal kinases; K_2HPO_4, dipotassium hydrogen phosphate; KCl, potassium chloride; KH_2PO_4, potassium dihydrogen phosphate; MCAo, middle cerebral artery occlusion; $MgCl_2$, magnesium chloride; Na_2HPO_4, disodium hydrogen phosphate; NaCl, sodium chloride; NADH, nicotinamide adenine dinucleotide reduced; NADPH, nicotinamide adenine dinucleotide phosphate; NaH_2PO_4, sodium dihydrogen phosphate; NaOH, sodium hydroxide; NED, naphthyl ethyl diamine; NF-kB, nuclear factor kappa beta; NM, nimodipine; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PMS, phenazonium methosulphate; PPAR-γ, peroxisome proliferator-activated receptor-γ; RH, relative humidity; ROS, reactive oxygen species; SD, Sprague Dawley; SO, sham operated; SSA, sulphosalicylic acid; TBA, thiobarbituric acid; TBARs, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TM, telmisartan; TNF-α, tumour necrosis factor-α; UV–Vis, ultra violet–visible.

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1. Introduction

Decreased cerebral blood flow mediated deprivation of oxygen, energy metabolites and micronutrients could increase intracellular cytosolic calcium level (Ca^{2+}), and release excitatory amino acids (EAAs), toxic free-radicals, and cytokines. It could also precipitate acidosis which might exacerbate the cerebral ischemia (Siesjo, 1992a, 1992b). Among the events, excitotoxicity plays a crucial role in the pathophysiology of cerebral ischemia. Glutamate is the major EAA in the brain, and produces neuronal injury and cell death during cerebral ischemic condition via Ca^{2+} dependent N-methyl-D-aspartate (NMDA) receptor signalling (Rothman and Olney, 1986). Glutamate induced excitotoxicity is controlled through clearance of glutamate at the synapse predominantly through GLT-1 (glutamate transporter-1) (Danbolt, 2001; Beart and O'Shea, 2007). Glutamate release and Ca^{2+} mediated toxicity occur simultaneously in the neurons during ischemic condition. Nimodipine, a central Ca^{2+} channel blocker (NM) was studied widely in this condition because of its blood brain barrier (BBB) permeability and cerebral vasodilatation property. Post-ischemic administration of NM restored the energy levels and protected the hippocampus and cortical regions in middle cerebral artery occluded (MCAo) rats (Babu and Ramanathan, 2009). NM exhibited anti-inflammatory activity through the control of cytokine levels in vascular dementia (Zhang et al., 2012), amyloid beta treated N13 microglial cell model (Sanz et al., 2012) and also in patients affected with cerebral haemorrhage (Zhicong et al., 2010). Li et al. (2009) have reported neuroprotection in dopamine neurons treated with NM and this effect was attributed due to inhibition of microglial mediated oxidative stress and inflammatory response.

Angiotensin receptor-1 (AT_1) blockers have shown neuroprotective role in ischemic state. Experimental findings have shown that pre-ischemic treatment of telmisartan (TM) (5 mg/kg) suppressed the cerebral injury in a murine model of transient focal ischemia and the effect was attributed to anti-inflammatory and antioxidant effects (Kasahara et al., 2010). Recent studies have shown that TM directly decreased the neuronal inflammatory response through attenuated interleukin-1β (IL-1β) expression and partial blockade of c-Jun N-terminal kinases (JNK/c-Jun) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathways in neuronal culture (Pang et al., 2012).

In preclinical studies, control of cytokine release or prevention of its action during ischemic state has exhibited neuroprotective effect. Among the cytokines, IL-1β elevation played a significant role in neuroprotection during ischemia. In clinical condition, IL-1β and tumour necrosis factor-α (TNF-α) are the major cytokines that exhibited inflammatory role in the patients affected by stroke. Recent studies have shown that the excess production of inflammatory cytokines during neurodegeneration impaired the glutamate uptake into the astrocytes as well as microglia that may be the key factor of neurodegeneration. Expression and function of GLT-1 by primary astrocytes were suppressed in vitro following exposure to IL-1β and TNF-α (Szymocha et al., 2000; Wang et al., 2003; Korn et al., 2005). Further, TNF-α is the key cytokine that stimulates extensive microglial glutamate release in an autocrine manner by up-regulating glutaminase to cause excitotoxicity (Takeuchi et al., 2006). Therefore it can be stated that control of cytokine release might prevent glutamate elevation during ischemia.

Hence, it is clear that many factors might contribute to the pathology of cerebral ischemia. Therefore, multiple approaches are required to protect neurons in cerebral ischemic condition. Hence, the present study was designed to evaluate the neuroprotective effect of non-hypotensive doses of TM (Kasahara et al., 2010) and its combination with NM in MCAo model. In post-ischemic state, the downstream responses are through multiple mechanisms, hence the effect of the drug in controlling EAA, cytokine, and energy levels was studied. A special emphasis has been given to understand the correlation of cytokines with EAA. The proposed additive effect of NM with TM on MCAo will give additional value in the treatment of stroke condition because both drugs control blood pressure; one of the major risk factors in precipitating stroke.

2. Materials and methods

2.1. Chemicals

TM and NM were gift samples from Zydus Cadila, Ahmedabad, India. Other chemicals were purchased: adenosine triphosphate (ATP) and naphthyl ethyl diamine (NED) (Sigma, St. Louis, USA); amino napthol sulphonic acid, glutamate, aspartate, thiobarbituric acid (TBA), dithiodinitrobenzene (DTNB), and dinitrophenyl hydrazine (DNPH) (Merck, Mumbai, India); sulphanilamide, sulphosalicylic acid (SSA), and Tris–HCl (HiMedia, Mumbai, India); and phenazonium methosulphate (PMS), nicotinamide adenine dinucleotide reduced (NADH) and butylated hydroxyl toluene (BHT) (SISCO Research Laboratories, Mumbai, India). Pre-coated HPTLC plates were purchased from Merck, Mumbai, India and 4-0 nylon monofilament was procured from Ethicon®, Chennai, India. All other chemicals, reagents and solvents were of analytical grade unless mentioned.

2.2. Animals

Male Sprague Dawley (SD) rats (each weighing 250–300 g and 6 months old) used in the study were supplied from central animal house facilities, PSG Institute of Medical Sciences and Research (Coimbatore, India). The animals were housed in individual polypropylene cages in a well-ventilated room under an ambient temperature of 25 ± 2 °C and 55% relative humidity (RH), with 12 h light/12 h dark artificial photoperiod. They were provided with standard rat food and purified water ad libitum. All the experimental animals were acclimatised for at least 7 days to laboratory conditions prior to experimentation. The guidelines from "Guide for the Care and Use of Laboratory Animals" (Indian Council of Medical Research) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC), PSG Institute of Medical Sciences and Research, Coimbatore, India approved the ethical aspects of the study (proposal authorisation number — 158/PO/BC/99/CPCSEA/168).

2.3. Experimental design and drug treatment

The rats were divided into five groups (each group, n = 9), the sham-operated (SO) and ischemic reperfusion (IR) rats received 0.3% carboxy methyl cellulose (CMC), whereas the test animals received TM 5 mg/kg (TM5), TM 10 mg/kg (TM10) or TM 5 mg/kg + NM 5 mg/kg (TM5 + NM5) combination. The dose of TM & NM and time of drug administration were based on earlier studies (Kasahara et al., 2010; Babu and Ramanathan, 2011). The drugs were suspended in 0.3% CMC and the treatment was started for all the groups 30 min prior to the surgery and continued once daily in the morning hours until the study was completed (Fig. 1).

2.4. Surgical procedure

Focal cerebral ischemia was induced by middle cerebral artery (MCA) occlusion as described by Babu and Ramanathan (2009). Rats were anaesthetised with chloral hydrate (350 mg/kg, i.p), and the physiological monitoring and physical monitoring of the rats were carried out to assure that deep anaesthesia was maintained. The parameters observed were abdominal respiration, non-retention of posture, and sensory evaluation in the paws by application of mild pressure. The right common carotid artery was exposed at the level of the external and internal carotid artery bifurcation. Nylon monofilament (4-0 size, Ethicon®) was used and its tip was made round headed by exposing it to flame. The filament was coated with 0.01% poly-L-Lysine and inserted