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Neuroprotection of agomelatine against cerebral ischemia/reperfusion injury through an antiapoptotic pathway in rat

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ABSTRACT

Agomelatine is an agonist of the melatonergic MT1/MT2 receptors and an antagonist of the serotonergic 5-HT receptors. Its actions mimic melatonin in antioxidative and anti-inflammation. However, the protective mechanism of agomelatine in ischemic/reperfusion (I/R) injury has not been investigated. In this study, cerebral I/R injury rats were induced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion. The rats were randomly divided into 6 groups (12 rats per group): sham-operated; vehicle-treated I/R; 20 mg/kg, 40 mg/kg, and 80 mg/kg agomelatine-treated I/R; and 10 mg/kg melatonin-treated I/R. Agomelatine and melatonin were intraperitoneally administrated to the rats 1 h before MCAO induction. After reperfusion for 24 h, the brain samples were harvested for evaluating the infarct volume, histological changes, terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining as well as cleaved caspase-3, Bax, Bcl-X_L, nuclear factor erythroid-2-related factor (Nrf2), and heme oxygenase (HO-1) levels. Agomelatine treatment significantly decreased apoptosis, with decreases in Bax and cleaved caspase-3, and increased Bcl-X_L, along with a decrease in apoptotic neuronal cells. Moreover, agomelatine was also found to markedly increase the expression of HO-1, the antioxidative enzymes, and the activity of superoxide dismutase (SOD) mediated by Nrf2 pathway. Agomelatine treatment protects the brain from cerebral I/R injury by suppressing apoptosis and agomelatine has antioxidant properties. Hence, there exists the possibility of developing agomelatine as a potential candidate for treating ischemic stroke.

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1. Introduction

Cerebral stroke is the most common cause of permanent disability and mortality in the world (Feigin et al., 2003). Ischemic stroke is the loss of neuronal function resulting from a reduction in cerebral blood flow (Lyden and Zivin, 1993; Kochanski et al., 2013; Li et al., 2014). Reperfusion is the primary treatment for ischemic stroke to restore blood flow. Recent evidences showed that reperfusion insults produce large amounts of reactive oxygen species (ROS) (Aguiar et al., 2013), such as superoxide, hydroxyl radical, hydrogen peroxide, etc., that cause brain damage via several mechanisms including glutamate excitotoxicity, inflammation, blood brain barrier (BBB) disruption, and, especially, oxidative stress subsequence cellular damage and apoptosis (Demirdas et al., 2016; Sarkar et al., 2016).

Oxidative stress following cerebral ischemia/reperfusion injury (I/R) has been known to lead to the disruption of physiological balance oxidants and antioxidants. The transcription factor nuclear factor erythroid-2-related factor (Nrf2) is a key protein that controls the redox state of the cells under oxidative stress and has been shown to be protective in many pathological conditions including I/R injury in the central nervous system (CNS). Under basal conditions, Nrf2 is bound to its inhibitors, such as the Kelch-like ECH-associated protein 1 (Keap1), which promote its proteosomal degradation. Oxidative stress leads to the dissociation of the Nrf2-

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Keap1 complex and promotes the translocation of Nrf2 into the nucleus. Nrf2 induces the expression of antioxidant enzymes, including glutathione-S-transferase (GST), heme oxygenase (HO-1), and NADH quinone oxidoreductase (NQO1), by binding to the antioxidant-responsive elements (AREs). Among them, HO-1 has been reported to have the most AREs on its promoter, making it a highly effective therapeutic target for protection against brain injury after an ischemic stroke. Nrf2 is a promising target to attenuate brain damage and neurological deficit following a stroke (Kang et al., 2005; Ding et al., 2014).

Strong evidence for neuronal apoptosis is observed in numerous I/R animal models. In the apoptotic pathway, the Bcl-2 family of proteins (e.g., anti-apoptotic Bcl-2 and Bcl-X_L; pro-apoptotic Bcl-Xs and Bax) and caspase (cysteinyl aspartate specific proteases) has been known to play an important role (Broughton et al., 2009). Experimental evidence indicates that both caspase-3 knock-out mice and Bcl-2 overexpression decreased ischemic infarction after MCAO (Lee and Won, 2014). Therefore, targeted inhibition of apoptosis pathway may provide an attractive therapeutic approach for the treatment of I/R injury.

Agomelatine is the first melatonergic antidepressant, an agonist of the melatonergic MT1/MT2 receptors and an antagonist of the serotonergic 5-HT receptors. Its actions mimic melatonin in the synchronization of circadian rhythm patterns in rodents (Levitan et al., 2015), and it has been shown to have protective effect against seizure in experimental models (Aguiar et al., 2013). The neuroprotective effects of melatonin on cerebral I/R brain injury in mice via increase anti-apoptotic protein and decrease apoptotic protein and partly via inhibition of the consequential inflammatory response were recently reported (Mauriz et al., 2013; Yang et al., 2015). Melatonin as well as its metabolites was also reported to be potent ROS scavengers (Yuruker et al., 2015; Kahya et al., 2016) which possess antioxidant and anti-inflammatory properties; in line with these evidences, we can speculate that agomelatine may act by the same pathway of melatonin to produce antioxidant effects. However, it remains unclear whether agomelatine exerts neuroprotection in the cerebral ischemic stroke model. Considering the important role of pathogenesis in cerebral stroke, elucidation of the effects of agomelatine may provide a new insight into its potential application for the prevention or treatment of cerebral ischemic stroke. Therefore, in this study, we investigated the possible mechanisms underlying the neuroprotective effects of agomelatine against cerebral I/R damage.

2. Materials and methods

2.1. Chemicals and reagents

Melatonin and 2,3,5-triphenyltetrazolium chloride (TTC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mouse anti-β-actin monoclonal, mouse anti-Bcl-X_L, mouse anti-Bax antibodies, anti-α-actin, anti-Nrf2, anti-HO-1, anti-mouse IgG peroxidase-conjugated secondary antibody, and anti-rabbit IgG peroxidase-conjugated secondary antibody were purchased from Merck Millipore (MA, USA). Commercial kits used for determining superoxide dismutase (SOD), GSH, GSH-Px were purchased from Cayman (Cayman Chemicals, Ann Arbor, MI, USA).

2.2. Animals

Male Wistar rats aged 2 months weighting 220–250 g were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. The rats were housed under a 12 h darkness–light cycle, under a maintained temperature (24 ± 1 °C). The animals were allowed free access to rodent diet and tap water. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guidelines prepared by Chiang Mai University for the care and use of laboratory animals.

2.3. Model of middle cerebral artery occlusion (MCAO)

Cerebral ischemia was induced through middle cerebral artery occlusion (MCAO) in the model using a modified intraluminal technique (Longa et al., 1989). The rats were anesthetized with zolitil (50 mg/kg, intraperitoneally) and xylazine (3 mg/kg, intraperitoneally). The common right carotid artery was exposed and isolated, while the middle cerebral artery was occluded by inserting a nylon monofilament (4–0) with the tip rounded into the internal carotid artery (ICA) through an external carotid artery stump for approximately 17–20 mm past the carotid artery bifurcation, until a slight resistance was felt, to occlude the middle cerebral artery (MCA). Reperfusion was done by gently removing the filament after 2 h of ischemia. After reperfusion, the wound was closed with sutures. The animals were then returned to their cage for 24 h. The sham-operated animals were treated identically, except that the middle cerebral arteries were not occluded after the neck incision.

2.4. Animal treatment

In this experiment, 72 rats were randomly divided into 6 groups (n = 12 for each group): a sham-operated group (sham); a vehicle-treated group (vehicle); a 20 mg/kg, a 40 mg/kg, and an 80 mg/kg agomelatine-treated group; and a 10 mg/kg melatonin-treated group (Molteni et al., 2013; Zheng et al., 2014; Demir Ozkay et al., 2015). The rats in the agomelatine and the melatonin groups were injected intraperitoneally 1 h before MCAO.

2.5. Measurement of regional cerebral blood flow by laser doppler flowmetry

Laser Doppler flowmetry (ADInstruments, Dunedin, New Zealand) was used to monitor regional cerebral blood flow (rCBF) in the territory of the middle cerebral artery (5 mm lateral and 1 mm posterior from bregma) during pre-ischemia, ischemia, and reperfusion to verify the success of the cerebral ischemia/reperfusion procedure.

2.6. Neurological evaluation

All the animals were examined for neurological deficits at 24 h after the onset of I/R according to the method of Longa et al. (1989). Neurological findings were scored on a five-point scale: no neurological deficits = 0; failure to extend opposite forepawfully = 1; contralateral circling = 2; brain damageof not being able to grip the wire mesh and thus falling on the contralateral side = 3; no spontaneous motor activity = 4.

2.7. Measurement of infarct volume

The animals were euthanized, and the brain samples were quickly collected and frozen in brain matrices (−20 °C). Then, the brain samples were subsequently sliced into five coronal sections beginning from the anterior tip of the frontal lobe (2 mm thick). The slices were then immersed in 2% of TTC for 30 min at 37 °C followed by fixation with 10% formalin. The images were recorded with a digital camera and the infarct volume was analyzed with program Image J. The percentage of the infarct volume was calculated by the following formula: ([total contralateral hemispheric volume]–

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