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Research Report

Neuroprotective effects of madecassoside against focal cerebral ischemia reperfusion injury in rats

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ABSTRACT

Madecassoside, a triterpenoid derivative isolated from *Centella asiatica*, exhibits anti-inflammatory and antioxidant activities. We investigated its neuroprotective effect against ischemia-reperfusion (I/R) injury in cerebral neurons in male Sprague-Dawley rats. Madecassoside (6, 12, or 24 mg/kg, i.v.) was administered 1 h after the start of reperfusion, and neurological deficit score and infarct volume were evaluated 24 h later. Neuronal apoptosis was assessed by performing terminal deoxynucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL) staining, and pathological brain damage was estimated by performing hematoxylin and eosin staining. Serum levels of malondialdehyde, superoxide dismutase activity, reduced glutathione levels, and nitric oxide levels were also determined. mRNA and protein expression of pro-inflammatory cytokines (Interleukin-1β/6, and tumor necrosis factor-α) were measured by real-time RT-PCR and ELISA, respectively; NF-κB p65 expression was determined by western blotting. Madecassoside significantly reduced brain infarct area, resolved neurological deficit, and ameliorated neuronal apoptosis. It also significantly reduced the levels of malondialdehyde and nitric oxide,

Abbreviations: AD, Alzheimer's disease; CA, *Centella asiatica*; CCA, common carotid artery; c-IAP, cellular inhibitor of apoptosis; Cox-2, cyclooxygenase-2; CPA, cyclophosphamide; ECA, external carotid artery; ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; HE, hematoxylin and eosin; ICA, internal carotid artery; I/R, ischemia/reperfusion; IL-1β/6, interleukin-1β/6; MA, madecassoside; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; NF-κB, nuclear factor-kappa B; NIM, nimodipine; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; PBS, phosphate-buffered saline; PD, Parkinson's disease; PGE2, prostaglandin E2; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; SOD, superoxide dismutase; TBA, thiobarbituric acid; TdT, terminal deoxynucleotidyl transferase; TLR, Toll-like receptors; TNF-α, tumor necrosis factor-α; TTC, 2,3,5-triphenyltetrazolium chloride; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling

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and augmented the antioxidant activity in rats subjected to cerebral I/R. Moreover, the levels of pro-inflammatory cytokines and NF-κB p65 significantly reduced after madecassoside treatment. These results indicate that madecassoside is neuroprotective and may be useful in reducing the damage caused by stroke.

1. Introduction

Ischemic stroke is one of the leading causes of human disability and mortality in the world (Lo et al., 2003). Restoration of an adequate blood flow is essential for survival. However, even after the restoration of blood flow, reperfusion itself causes tissue damage beyond that caused by ischemia through mechanisms that include excitotoxicity, oxidative stress, inflammation, and apoptosis. Although cerebral ischemia-reperfusion (I/R) injury has been extensively studied in the laboratory, few neuroprotective strategies have successfully translated from basic science to the clinic.

A lot of attention in the field of drug discovery has been focused on the neuroprotection attributed to natural compounds from traditional medicinal herbs, because the compounds with multiple targets appear to be a potential new and promising class of therapeutics for the treatment of neurological disorders with a multifactorial etiology, including ischemic brain damage(An et al., 2011; Kim et al., 2007; Kumar and Khanum, 2012; Loh et al., 2010; Wu et al., 2010). *Centella asiatica* (CA), a tropical medicinal plant of the Apiaceae family, grows in Southeast Asian countries, such as China, India, and Malaysia, and in South Africa and Madagascar. CA extracts exhibit a wide range of pharmacological activities, including wound healing (Ermertcan et al., 2008; Somboonwong et al., 2012), scar management (Bian et al., 2013), and anticancer activity (Babykutty et al., 2008; Hussin et al., 2014). Of particular relevance to the present study are the findings of several studies that have shown that CA extracts can improve central nervous system function as an anxiolytic (Jana et al., 2010; Wanasuntronwong et al., 2012; Wijeweera et al., 2006), antidepressant (Chen et al., 2005), anticonvulsant (Visweswari et al., 2010), memory enhancer (Kumar et al., 2009; Meena et al., 2012; Xu et al., 2012), and neuroprotector in models of Alzheimer's Disease (AD) (Dhanasekaran et al., 2009; Soumyanath et al., 2012) and Parkinson's disease (PD) (Xu et al., 2013). Notably, CA has been reported to significantly improve neurobehavioral activity and reduce infarct volumes in rats subjected to middle cerebral artery occlusion (MCAO). The remarkable activities of CA may be attributed to its bioactive triterpenes, asiatic acid, asiaticoside, madecassic acid, and madecassoside (MA) (Hashim et al., 2011), but the constituent responsible for this effect remains unknown (Tabassum et al., 2013).

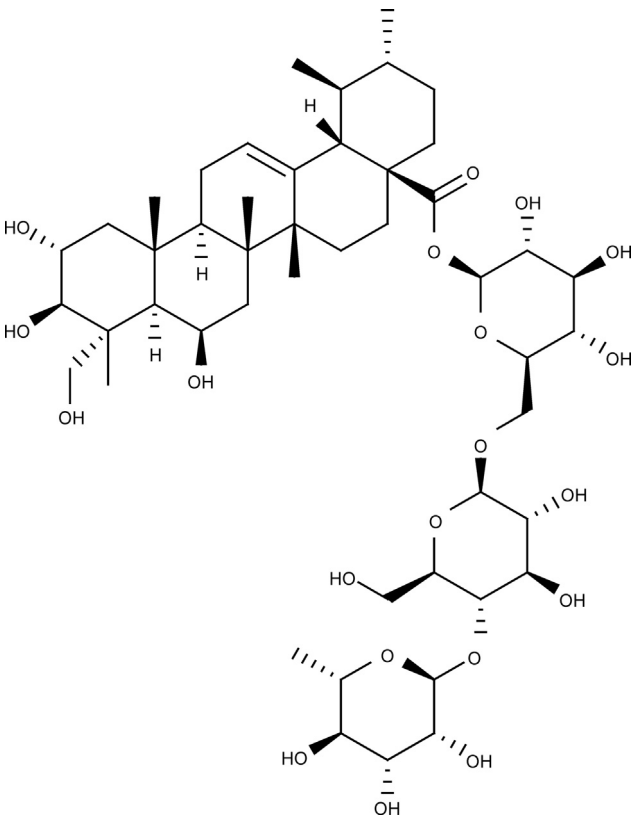

Fig. 1 – Chemical structure of madecassoside.

MA (Fig. 1), the major pentacyclic triterpenoid saponin component of CA, has been characterized as a potential anti-inflammatory agent (Li et al., 2009; Liu et al., 2008b; Wu et al., 2012). MA has also been reported to reduce myocardial I/R injury (Bian et al., 2008). In light of the similarities in the pathologic mechanisms underlying both cerebral and myocardial ischemia, we hypothesized that MA can alleviate damage caused by cerebral I/R injury. Thus, the aim of this study was to investigate the therapeutic efficacy of MA on focal cerebral I/R-induced impairments in rats. We evaluated the infarct size and neurologic deficits in MA-treated rats in comparison with those in the saline-treated animals. Studies were also designed to determine the mechanism of action of MA by investigating its anti-oxidant, anti-inflammatory, and anti-apoptotic functions, with a particular focus on the NF-κB pathway.

2. Results

2.1. MA decreased neurologic deficits

Neurological deficits were evaluated 24 h after the start of reperfusion. Sham-operated controls exhibited no neurological deficits, whereas vehicle-treated rats had the highest neurological deficit score. Neurological deficit score for animals treated with a combination of nimodipine (NIM) and cyclophosphamide (CPA), which was used as a comparative