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Magnolol protects neurons against ischemia injury via the downregulation of p38/MAPK, CHOP and nitrotyrosine

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ABSTRACT

Magnolol is isolated from the herb *Magnolia officinalis*, which has been demonstrated to exert pharmacological effects. Our aim was to investigate whether magnolol is able to act as an anti-inflammatory agent that brings about neuroprotection using a global ischemic stroke model and to determine the mechanisms involved. Rats were treated with and without magnolol after ischemia reperfusion brain injury by occlusion of the two common carotid arteries. The inflammatory cytokine production in serum and the volume of infarction in the brain were measured. The proteins present in the brains obtained from the stroke animal model (SAM) and control animal groups with and without magnolol treatment were compared. Magnolol reduces the total infarcted volume by 15% and 30% at dosages of 10 and 30 mg/kg, respectively, compared to the untreated SAM group. The levels of acute inflammatory cytokines, including interleukin-1 beta, tumor necrosis factor alpha, and interleukin-6 were attenuated by magnolol. Magnolol was also able to suppress the production of nitrotyrosine, 4-hydroxy-2-nonenal (4-HNE), inducible NO synthase (iNOS), various phosphorylated p38 mitogen-activated protein kinases and various C/EBP homologues. Furthermore, this modulation of ischemia injury factors in the SAM model group treated with magnolol seems to result from a suppression of reactive oxygen species production and the upregulation of p-Akt and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). These findings confirm the anti-oxidative properties of magnolol, including the inhibition of ischemic injury to neurons; this protective effect seems to involve changes in the *in vivo* activity of Akt, GSK3β and NF-κB.

Introduction

Magnolol, a major active agent isolated from the herb *Magnolia officinalis* (Chinese herbal name: *Hou Pu*) is a poly-phenolic compound that exerts its biological properties through a variety of mechanisms (Alonso-Castro et al., 2011; Domínguez et al., 2010). Magnolol has been extensively documented and possesses a range of therapeutic properties such as anxiolytic activity (Kuribara et al., 2000), analgesic activity (Lin et al., 2009), antidepressant activity (Qiang et al., 2009), antimicrobial activity (Kim et al., 2010), antispasmodic activity (Ko et al., 2003), anti-tumorigenic activity (Chen et al., 2009) and an anti-Alzheimer's therapeutic effect (Hoi et al., 2010). Importantly, a recent study has indicated that oral magnolol seems to prevent age-related memory and learning deficits found in senescence-accelerated mice and does this by preserving cholinergic neurons (Lin et al., 2006; Matsui et al., 2009a,b). However, the mechanism by which the magnolol initiates neuroprotection against ischemic injury to the brain remains poorly understood.

Stroke is the fourth-leading cause of death and the primary cause of long-term disability worldwide (Iadecola and Anrather, 2011). It involves a distinct sequence of events that are not yet fully elucidated. Ischemic insult induces excessive generation of free radicals (reactive oxygen species (ROS) and reactive nitrogen species (RNS)), which then cause endoplasmic reticulum stress, which is signaled by both increased circulating levels of proinflammatory cytokines (TNF, IL-6) and the induction of macrophage infiltration that has been linked to apoptosis by the inducible NOS (iNOS) activation; these events then induce apoptotic cell death in neurons (Towfighi and Saver, 2011). Further ischemic stroke events also occur and these include neurotoxicity

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mediated by the production of free radicals and a subsequent induction of the p38 MAPK/CHOP pathway that is involved in ER stress-induced apoptosis of neurons; these findings are supported by the fact that ischemic injury is reduced following the induction of endogenous antioxidant pathways and the presence of scavengers targeting nitric oxide (Gotoh and Mori, 2006). As a result of the above findings, a fundamental role as potential neuroprotective agents is recognized for free radical scavengers. This is because ischemic injury is caused by a series of events that involve energy depletion and cell death and these are mediated by various intermediate factors including excess extracellular excitatory free radical formation and the presence of inflammation (Kohno et al., 1997). After arterial occlusion, necrotic cell death occurs predominantly in the ischemic core (Iadecola and Anrather, 2011). Reduced blood flow resulting from arterial occlusion or hypotension leads to tissue hypoxia and hypoglycemia, which then cause protein misfolding and endoplasmic reticulum stress. Ischemia-reperfusion injury of the brain then induces oxidative stress, which leads to production of nitric oxide (NO), a mediator of protein nitrosylation, and other reactive oxygen species (ROS); these alter cellular redox-dependent reactions and have an effect on protein misfolding (Zhu et al., 2003). Despite efforts to develop novel drugs to rescue neurons from delayed neuronal death in the penumbral region of the ischemic core, few currently available drugs are able to effectively treat stroke patients. In the present study we shall explore the biological agent of magnolol and its effect on ischemia reperfusion injury.

Reactive oxygen species derived from ischemia-reperfusion have been shown to be associated with the phosphatidylinositol 3-kinase (PI3K) and Akt signaling pathway that leads to neuronal survival or death (Noshita et al., 2001). PI3K/Akt is a major cell survival pathway that has been extensively studied. The PI3K/Akt pathway promotes cellular survival and cell cycling by phosphorylating and inhibiting death-inducing proteins; this includes phosphorylation of glycogen synthase kinase-3 beta (GSK-3β), and various activation-associated nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent prosurvival genes (Camandola and Mattson, 2007). Considering the key role of PI3K/Akt and NF-κB in cell survival when studied using a stroke model of ischemia reperfusion brain injury, in the present study we sought to determine whether magnolol is able to effectively improve the neuroprotective effects of PI3K/Akt and NF-κB.

In our current study, a cerebral global ischemic stroke model of temporary ischemia followed by reperfusion onset was used to assess whether magnolol has a neuroprotective effect. We were able to demonstrate that magnolol, a known antioxidant, is able to reduce the oxidative endoplasmic reticulum stress that is generated by global cerebral ischemic injury, thus promoting neuronal cells survival; this involved the p38 mitogen-activated protein kinases (MAPKs), C/EBP homologous protein (CHOP) and the Akt/ NF-κB signal pathway.

Material and methods

Surgery to induce ischemia reperfusion brain injury and drug administration. Adult male Sprague–Dawley rats weighing around 280 ± 20 g were kept individually in a 12-hour light/dark cycle cage and had free access to water and food. Animal care and the general protocols for animal use were approved by the Institutional Animal Care and Use Committee of National Yang-Ming University. All efforts were made to minimize the number of animals used and their suffering. These rats were operated on according to the modified global cerebral ischemia's model, which involves occlusion of the two common carotid arteries, that is the model uses two vessel occlusion (2VO) to induce reversible ischemia for a limited time period (Smith et al., 2007). In brief, the bilateral carotid arteries to be occluded were ligated with 4-0 nylon under 10% chloral hydrate anesthesia (350 mg/kg, injection with 1 ml/kg of the solution). These filaments were withdrawn 90 min after the onset of ischemia. The femoral artery and vein were exposed and cannulated with PE-50 polyethylene tubing (Fisher Scientific) (Smith et al., 2007). The arterial catheter was used for continuous blood pressure recording and blood gas analysis (AVL 990; Homburg, Germany). Body temperature was maintained at 37 °C via a homoeothermic blanket. Sham-operated

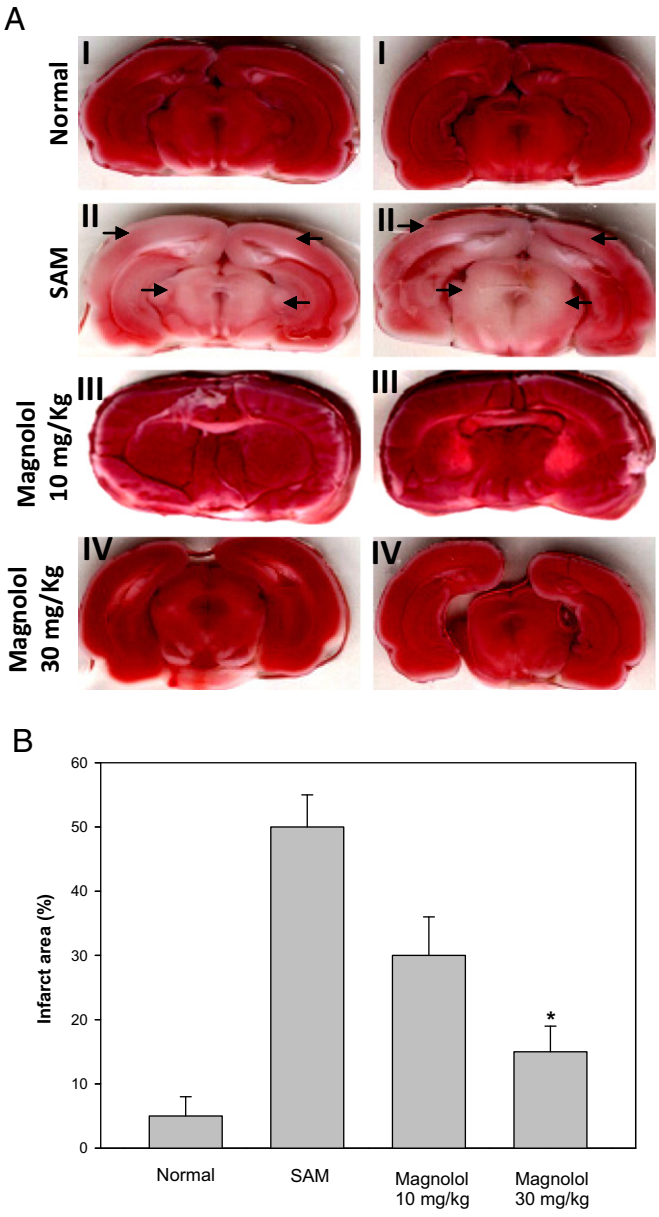

Fig. 1. Effects of magnolol on the infarct volume using an ischemic rat model. The stroke animal model (SAM rat 1.5 h and reperfusion 24 h) used rats as described in 'Material and methods'. Magnolol was administered intraperitoneally after initial of ischemia. The sham-operated control group and the SAM group animals received an equal volume of solvent. (A) Representative ischemic lesions, as assessed by TTC staining, present in 2 mm thick coronal sections from the brains of rats treated with magnolol at the dose of 10 and 30 mg/kg (injection at 90 min post-stroke rat) 1 day after ischemia and from the brains of rats that received DMSO only 1 day after ischemia. Infarct volume was assessed at 14 days after arterial occlusion. Ischemic injury is indicated by the filled arrowheads in the contralateral cortex and subcortex. Representative stained brain sections from the sham-operated control group rats (I), the untreated rats with transient SAM (II), the rats with transient SAM treated with magnolol (10 mg/kg) (III) and the rats with transient SAM treated with magnolol (30 mg/kg) (IV). (B) Quantitative analysis of total infarct volume showing the therapeutic effect of magnolol; this was done by comparing the three groups of six with transient SAM rats with and without magnolol treatment at 24 h post-stroke rat. Cortex, subcortex, and total infarct volumes were determined for the transient stroke rats (shown as percentage of the hemisphere). Data are expressed as mean ± SD of independent experiments. $^{*}p < 0.05$, SAM group versus SAM + magnolol group. $^{\#}p < 0.05$, SAM group versus SAM + magnolol group.