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Preventive effect of silymarin in cerebral ischemia–reperfusion-induced brain injury in rats possibly through impairing NF-κB and STAT-1 activation

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ABSTRACT

Silymarin and silibinin are bioactive components isolated from *Silybum marianum*. They have been reported to exhibit anti-oxidative and anti-inflammatory effects. Many studies revealed that drugs with potent anti-inflammatory potential can protect animals against inflammation-associated neurodegenerative disease, e.g., stroke. In this current work we established an animal model of acute ischemic stroke injury by inducing cerebral ischemic/reperfusion (CI/R) in rats to elucidate whether silymarin or silibinin can protect animals from CI/R injury. Pretreatment with silymarin, but not silibinin, dose-dependently (1–10 μg/kg, i.v.) reduced CI/R-induced brain infarction by 16–40% and improved neurological deficits in rats with a stroke. Elevated pathophysiological biomarkers for CI/R-induced brain injury, including lipid peroxidation, protein nitrosylation, and oxidative stress, were all reduced by silymarin. In addition, expression of inflammation-associated proteins (e.g., inducible nitric oxide synthase, cyclooxygenase-2 and myeloperoxidase), and transcriptional factors (e.g., nuclear factor (NF)-kappa B and signal transducer and activator of transcription (STAT)-1), as well as production of proinflammatory cytokine (e.g., interleukin-1β and tumor necrosis factor-α) was all significantly prevented by silymarin. Furthermore, an *in vitro* study on microglial BV2 cells showed that silymarin could inhibit nitric oxide and superoxide anion production, possibly by interfering with NF-κB nuclear translocation/activation. Likewise, silymarin pretreatment also inhibited IκB-α degradation and NF-κB nuclear translocation in brain tissues of ischemic rats. Our results reveal that silymarin, but not its active component silibinin, protected rats against CI/R-induced stroke injury by amelioration of the oxidative and nitrosative stresses and inflammation-mediated tissue injury through impeding the activation of proinflammatory transcription factors (e.g., NF-κB and STAT-1) in the upregulation of proinflammatory proteins and cytokines in stroke-damaged sites. In conclusion, silymarin displays beneficial effects of preventing inflammation-related neurodegenerative disease, e.g., stroke, which needs further investigation and clinical evidences.

Introduction

Ischemic stroke is one of the important causes of death in industrialized countries with a high incidence affecting up to 0.2% of the population each year (Klijn and Hankey 2003). The major pathological mechanism leading to ischemic/reperfusion brain injury during ischemic stroke is the so-called "excitotoxicity", an inappropriate activation of ionotropic *N*-methyl-D-aspartate (NMDA) receptors in the brain by excessive released glutamate which accumulates in the extracellular space after stroke onset. Exci-

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totoxicity is destructive which excites neurons to death through inducing overproduction of reactive oxygen species (ROS) [e.g., hydroxyl radicals (OH^-), superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2)] and reactive nitrogen species (RNS) [e.g., nitric oxide (NO) and peroxynitrite ($OONO^-$)], the so-called oxidative and nitrosative stresses. These free radicals may be produced by many of the free radical producing-enzyme systems including mitochondria, cyclooxygenase (COX), xanthine oxidase, NADPH oxidase (NOX), and inducible nitric oxide synthase (iNOS) in response to the activation of proinflammatory mediators produced by recruited leukocytes (e.g., neutrophils), active microglial cells, damaged neurons and astrocytes in stroke-injured tissues (Lo et al. 2003; Madrigal et al. 2004; Shen et al. 2008).

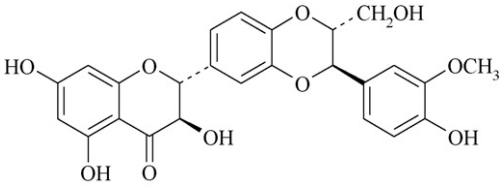

Fig. 1. Chemical structure of silibinin (silybin).

ROS and RNS damage tissues by attacking DNA or inducing lipid peroxidation or protein nitrosylation of the cell membrane and organelles (Lo et al. 2003). An inflammatory cascade is therefore initiated in tissue injured by ROS/RNS, leading to leukocyte infiltra-

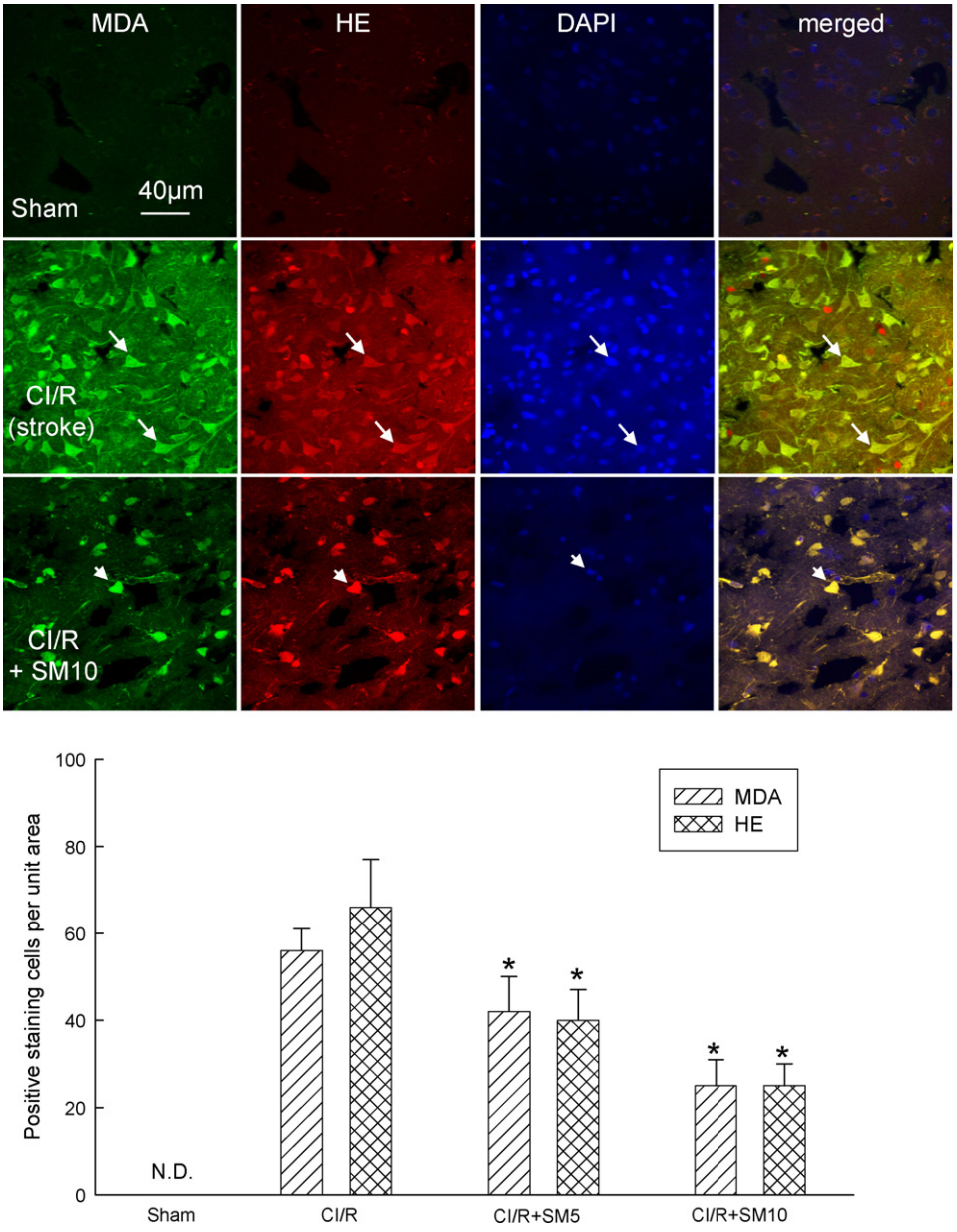

Fig. 2. Effects of silymarin pretreatment on changes in malondialdehyde (MDA) and superoxide formation at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. (Upper panel) Confocal images of MDA formation (green) and a superoxide marker (oxidized dihydroethidium (HE, red)) in the ipsilateral cerebral cortex. The nuclei of these cells were visualized by DAPI staining (blue). Arrows indicate the colocalization (yellow) of green fluorescence and red fluorescence (HE) in the merged columns. Pretreatment with silymarin (5 or 10 μg/kg, i.v.; CI/R + SM5 or CI/R + SM10) significantly reduced the staining of MDA and HE. (Lower panel) Statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. $^*p < 0.05$, compared with the corresponding CI/R group only by one-way ANOVA followed by Student–Newman–Keuls *t*-test. N.D., not detectable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)