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Further pharmacological evidence of the neuroprotective effect of catalpol from *Rehmannia glutinosa*

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Abstract

We have previously evaluated the neuroprotective effect of catalpol on aging mice induced by D-galactose, in which catalpol treatment ameliorated cognition deficits and attenuated oxidative damage in mice brain. To thoroughly elucidate the anti-aging effects of catalpol, the liver and spleen antioxidative systems and energy metabolism in senescent mice induced by D-galactose have been studied. Except control group, mice were subcutaneously injected with D-galactose (150 mg kg^{-1} body weight) for 6 weeks. Meanwhile, drug group mice were treated with catalpol (2.5, 5, 10 mg kg^{-1} body weight) and piracetam (300 mg kg^{-1} body weight) for the last 2 weeks. The activities of endogenous antioxidants and the level of glutathione (GSH) and lipid peroxide in the liver and spleen were assayed. Compared to control group, model group mice had significantly lower spleen index (spleen weight/body weight), lower level of GSH, lower activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX), higher level of malondialdehyde (MDA) in the liver and spleen. However, catalpol administration markedly reversed these effects of senescence induced by D-galactose. Simultaneously, catalpol noticeably elevated the decreased activities of lactate dehydrogenase (LDH), glutamine synthetase (GS), Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase and decreased the elevated activity of creatine kinase (CK) in mice liver or spleen. These results implied that the anti-aging effects of catalpol were achieved at least partly by promoting endogenous antioxidant enzyme activities and normalizing energy disturbance. Catalpol may be a potential anti-aging agent and worth testing for further preclinical study aimed for senescence or neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Keywords: *Rehmannia*; Catalpol; Antioxidant and antiaging effect; Neuroprotection

Introduction

Rehmannia, known as *dihuang* and disui in Shennong Bencao Jing (ca. 100 A.D.), refers to the root of *Rehmannia glutinosa*, an herb of the Scrophulariaceae family. *Rehmannia* is far more frequently prescribed in China than in other countries and has been used to replenish vitality, strengthen the liver, kidney, heart and for treatment of a variety of ailments like diabetes, anemia, and urinary tract problems, especially to be used in cases of kidney yin deficiency that is associated with aging. Kidney yin deficiency is regarded as the origin of age-related memory loss in Chinese traditional medicine. Therefore, *Rehmannia* is now more often used to treat age-related disorders, such as aging and dementia. Rehmanniae Decoction of Six Ingredients has long been used in age-related diseases and its therapeutic efficacy has been confirmed by many studies. Chronic administration of Rehmanniae Decoction of Six Ingredients to senescence-accelerated mouse promoted the

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spatial memory ability in water maze test and partially improved the learning behavior in conditioned avoidance performance (Zhou et al., 1999; Cui et al., 2004).

In our search for active ingredients, catalpol (Fig. 1), an iridoid glycoside, was isolated from fresh root of *Rehmannia* with column chromatography method. Catalpol possesses many therapeutic effects such as anti-inflammatory, promotion of sex hormones production, reduction of bleeding, protection of liver damage, and reduction of elevated blood sugar. Our previous works have demonstrated that catalpol can protect against H_2O_2-induced oxidative damage on PC12 cells and LPS-induced neurotoxicity on dopaminergic neurons (Jiang et al., 2004; Tian et al., 2006). In animal models, we found that catalpol could enhance cognitive performance in transient global ischemia in gerbils and protect mice brain from oxidative damage and mitochondrial dysfunction induced by rotenone (Li et al., 2004; Mao et al., 2007). Most important is that catalpol could increase presynaptic proteins and up-regulate relative signaling molecules in the hippocampus of the aged rats (Liu et al., 2006). Considering these neuroprotective effects of catalpol and the anti-aging effect of *Rehmannia*, we speculate that catalpol may be the main anti-aging active component of this Chinese crude drug. So we use aged animal model to verify the anti-aging effect of catalpol and the mechanisms underlying its effect.

These days, the ''free radical theory of aging'' is showing promise in helping to understand the process of aging and in finding effective anti-aging agents. The theory postulates that the generation of reactive oxygen species (ROS) or free radicals can lead to cell and tissue damage paralleled by alterations in the function of the genetic apparatus, resulting in aging and untimely cell death (Harman, 1956). To protect cells against oxidative damage produced by oxidants during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organisms. Enzymatic antioxidants, like superoxide dismutase (SOD) or glutathione peroxidase (GSH-PX), as well as non-enzymatic antioxidants like glutathione (GSH), can scavenge free radicals or prevent their formation. Lipid peroxidation plays an important role in tissue injury. Malondialdehyde (MDA), a stable metabolite of the free radical-mediated lipid peroxidation cascade, is widely used as marker for oxidative stress.

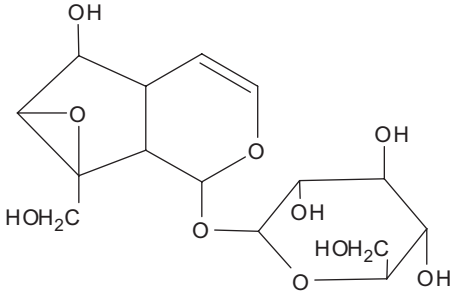

Fig. 1. Chemical structure of catalpol.

Furthermore, aging also results in energy metabolism failure with decrease in adenosine triphosphate (ATP) formation (Tian et al., 2005) and activity of lactate dehydrogenase (LDH) and excessive accumulation of lactic acid (Yue et al., 1999). Previous studies have demonstrated that oxidative processes often result in changes of the activities of key enzymes, including glutamine synthetase (GS), creatine kinase (CK) and tyrosine hydroxylase (Hensley et al., 1995). These changes result in a proceeding loss of cellular function which is regarded as a main reason for aging (De Grey, 1997).

Rodent chronically injected with D-galactose has been used as an animal aging model for anti-aging pharmacology research (Wei et al., 2005). Recently, we found that catalpol could enhance cognitive performance in aged mice induced by D-galactose and reduce oxidative stress by increasing the activities of SOD and GSH-Px and decreasing the concentration of MDA in mice brain (Zhang et al., 2007). These indicated that catalpol may be of potential use in the therapy of anti-aging. The aim of this study was to investigate the potential role of catalpol in reducing oxidative stress in the liver and spleen of senescent mice induced by D-galactose by quantifying lipid peroxidation and various antioxidant enzyme activities. In addition, we intended to explore the effect of catalpol on mice liver or spleen energy metabolism via detecting the activities of LDH, CK, GS, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase.

Materials and methods

Reagents and drugs

D-galactose was purchased from Shanghai Yuanju Chemical-Regent Company (Shanghai, China) and dissolved in 0.9% saline at concentrations of 3%. Catalpol (separation process see Preparation of catalpol) and piracetam (Pingyuan pharmaceutical factory, Shandong, China) were dissolved in physiological saline. Commercial kits used for determination of MDA, SOD, GSH, GSH-Px, LDH, CK, GS, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase were purchased from Jiancheng Institute of Biotechnology (Nanjing, China).

Preparation of catalpol

Fresh root of *Rehmannia* (1 kg) were homogenated and extracted three times at room temperature with 95% EtOH (each 5 l) for 24 h. The extracts were filtered and evaporated under reduced pressure (50 °C,