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Ginkgo biloba leaf extract and alpha-tocopherol attenuate haloperidol-induced orofacial dyskinesia in rats: Possible implication of antiapoptotic mechanisms by preventing Bcl-2 decrease and Bax elevation

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ABSTRACT

Background: Tardive dyskinesia (TD) is a serious side effect of long-term administration of typical neuroleptics, such as haloperidol. The pathophysiology of TD remains unclear, but the experimental evidence suggests that free radical-induced neuronal apoptosis in the basal ganglia may play an important role.
Purpose: This study was to investigate changes in Bax and Bcl-2 expression levels in TD-associated brain regions and the effects of the antioxidant EGb761 on Bax and Bcl-2 levels in an animal model of TD.
Methods: Thirty-two rats were randomly divided into four study groups: saline control (saline), haloperidol-alone (haloperidol), EGb761-haloperidol (EGb), and alpha-tocopherol-haloperidol (vitamin E). Rats were treated with daily intraperitoneal haloperidol injections (2 mg/kg/day) for 5 weeks. EGb761 (50 mg/kg/day) and alpha-tocopherol (20 mg/kg/day) were then administered for another 5 weeks during the withdrawal period. Behavioral assessments were performed, and Bax and Bcl-2 protein expression levels were immunohistochemically analyzed in four brain regions, including the prefrontal cortex, striatum, substantia nigra, and globus pallidum.
Results: We found that increased vacuous chewing movements (VCMs) were associated with increased proapoptotic Bax protein expression, decreased antiapoptotic Bcl-2 protein expression, and an increased Bax/Bcl-2 ratio. EGb761 and alpha-tocopherol treatment reversed the increase in VCMs, decreased Bax expression, increased Bcl-2 expression, and decreased the Bax/Bcl-2 ratio.
Conclusions: These results demonstrate that long-term haloperidol administration may affect Bcl-2 protein family expression and promote neuronal apoptosis in the basal ganglia. In combination with their antioxidant capacity, EGb761 and alpha-tocopherol's antiapoptotic effects through Bcl-2 might account for the symptom improvement observed in haloperidol-induced TD rats.

Introduction

Tardive dyskinesia (TD), which occurs in 20–50% of patients who receive chronic neuroleptic treatment, is characterized by repetitive involuntary movements that involve the mouth, face, tongue, and sometimes the limbs and trunk musculature. TD persists after drug withdrawal, indicating that antipsychotic drugs produce irreversible changes in the brain, especially in the basal ganglia (Casey, 2000; Egan et al., 1997; Howland, 2011; Kulkarni and Naidu, 2001; Trevizol et al., 2011). Although the relationship between TD and long-term antipsychotic treatment has been established, the pathophysiological basis of TD is poorly understood.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance (ANOVA); BDNF, brain-derived neurotrophic factor; CAT, catalase; EGb761, *Ginkgo biloba* extract; GABA, gamma aminobutyric acid; GSH, glutathione; HAL, haloperidol; IP, intraperitoneal injection; OD, optical density; ROI, regions of interest; ROS, reactive oxygen species; SD, Sprague Dawley; SOD, superoxide dismutase; TD, tardive dyskinesia; VCMs, Vacuous chewing movements; vitamin E, alpha-tocopherol.

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Several hypotheses have been proposed to explain the development of TD, including dopamine supersensitivity (Burt et al., 1977; Klawans and Rubovits, 1972; Tarsy and Baldessarini, 1974), dopamine D1/D2 receptor imbalance, gamma aminobutyric acid (GABA) deficiency (Gale, 1980), and the free radical hypothesis (Cadet and Lohr, 1989; Coyle and Puttfarcken, 1993; Floyd, 1999; Kulkarni and Naidu, 2001; Lohr and Bracha, 1988; Naidu and Kulkarni, 2001). Increasing evidence suggests that the neurotoxic overproduction and accumulation of free radicals induced by antipsychotic agents can damage the antioxidant defense system, resulting in oxidative stress and lipid peroxidation and thereby leading to potentially irreversible damage in motor regions (e.g., basal ganglia), which may be an underlying mechanism of the development of TD (Boskovic et al., 2011; Macedo et al., 2011). However, the precise mechanisms of free radical-induced neurodegeneration and neuronal apoptosis remain unknown, and little research on *in vivo* neuronal apoptosis has been performed.

Haloperidol (HAL), a typical neuroleptic, reportedly causes neuronal apoptosis *in vitro* (Galili et al., 2000; Noh et al., 2000; Ukai et al., 2004) and *in vivo* (Mitchell et al., 2002). The B-cell lymphoma 2 (Bcl-2) family of proto-oncogenes encodes specific proteins that regulate programmed cell death under different physiological and pathological conditions (Davies, 1995; Merry and Korsmeyer, 1997). Bcl-2 family members demonstrate anti- and proapoptotic functions: the major antiapoptotic family members include Bcl-2 and Bcl-x_L, and the major proapoptotic family members include BCL2-associated X protein (Bax) and Bcl-x_S (Adams and Cory, 1998). Proapoptotic molecules have the ability to form homo- and heterodimers with other proteins that regulate cell death, thereby modulating cell death in response to different paradigms (Yin et al., 1994). Generally, it is considered that Bcl-2 decreases and Bax increases in neurons entering apoptosis *in vivo* (Merry and Korsmeyer, 1997). Therefore, the Bax/Bcl-2 ratio can be used to reflect sensitivity to apoptosis.

The long-term administration of neuroleptics reportedly depletes the antioxidant glutathione (Sagara, 1998; Shivakumar and Ravindranath, 1993; Yokoyama et al., 1998) and endogenous antioxidant enzymes [catalase (CAT) and superoxide dismutase (SOD)]and elevates lipid peroxidation and reactive oxygen species (ROS) in various brain regions in animals models (Abilio et al., 2004; Dirican et al., 2007; Tsai et al., 1998). Similar results have been reported in HAL-treated patients (Lohr and Bracha, 1988). Antioxidants and free radical scavengers reportedly benefit the prevention and treatment of TD in animal models (Abilio et al., 2003; Abilio et al., 2002; Burger et al., 2003; Naidu and Kulkarni, 2004; Naidu et al., 2003a; Raghavendra et al., 2001; Sachdev et al., 1999) and humans (Dannon et al., 1997; Lohr et al., 2003).

In addition, evidence suggests that TD is associated with neuronal apoptosis, especially in the basal ganglia and subcortical parts of the brain (Boskovic et al., 2011; Macedo et al., 2011). In order to further elucidate the role of HAL-induced neuronal apoptosis in TD pathogenesis and the possible protective role of antioxidants, this study investigates changes in the expression of Bax and Bcl-2 in TD-related brain regions, including the prefrontal cortex, striatum, substantia nigra, and globus pallidum, and the effects of the antioxidants *Ginkgo biloba* extract (EGb761) and alpha-tocopherol on the expression of Bax and Bcl-2 in an animal model of TD.

Materials and methods

Animals

Male Sprague Dawley (SD) rats (180–220 g) were obtained from the Central Animal House of Beijing University of Chinese Medicine. All animals were housed under standard laboratory conditions, including a normal light-dark cycle and free access to food and water. The experimental protocols were approved by the institutional animal ethics committee and conducted according to the guidelines of the China National Science Academy for the use and care of experimental animals.

Drugs

Haloperidol (HAL) (Shanghai Jiufu Pharmaceutical Co., Ltd., China) was diluted with normal saline (NS) to the concentration of 1 mg/ml; alpha-tocopherol (Shanghai Roche Pharmaceutical Co., Ltd., China), it contains not less than 96.0% and not more than 102% d-alpha-tocopherol.

Preparation of EGb761

Ginkgo biloba L. extract (EGb761) was provided by Beijing University of Chinese Medicine, which was complying with the monograph in Chinese Pharmacopoeia Commission (2010). It was the 100% genuine dry extract from *Ginkgo biloba* L. leaf with 50% ethanol (v/v) as extraction solvent. The drug-extract ratio (DER) was within 50–56:1. The extract was quantitated using the methods in Chinese Pharmacopoeia Commission (2010), showing that the EGb761 contains 24.52% (w/w) flavonoid glycosides and 6.01% (w/w) terpene lactones, which were consistent with the previous reports (DeFeudis and Drieu, 2000; Xie et al., 2014). EGb761 was diluted with NS to the appropriate concentration. All compounds were administered orally in a constant volume of 5 ml/kg of rat body weight. The detailed analysis of EGb761 by high performance liquid chromatography (HPLC) was provided in the Supplementary File 1.

Measurement of vacuous chewing movements

Vacuous chewing movements (VCMs) are defined as a single mouth opening in the vertical plane that is not directed toward physical material (not including periods of grooming). Using the criteria of Naidu and Kulkarni (2001), three types of oral behavior were recorded: vertical jaw movements (each vertical opening and closing of the jaw was regarded as 1 VCM), bursts of jaw tremors, and tongue protrusions. Each burst of jaw tremors was regarded as equal to two VCMs. VCM consisted of rapid jaw movements that resembled chewing but did not appear directed at any particular stimulus. In addition, counting was stopped whenever the rat began grooming and restarted when grooming stopped. All VCM observations were made by one observer who was blinded to the treatment conditions.

In a preliminary study, we confirmed that VCM gradually increased with intraperitoneal injections of HAL (2 mg/kg/day) and plateaued in 5 weeks (An et al., 2013). Therefore, in the present study, the TD animal model was established using 5 weeks of intraperitoneal haloperidol injections (2 mg/kg/day).

Treatment schedule

Thirty-two rats were randomly divided into 4 groups with 8 animals each: (1) saline control group (saline), rats were intraperitoneally injected with saline for 5 weeks and then administrated saline by oral gavage for the following 5 weeks; (2) HAL-alone group (HAL), rats were injected with HAL (2 mg/kg/day) for 5 weeks and then administrated saline for the following 5 weeks; (3) alpha-tocopherol-HAL group (vitamin E), rats were injected with HAL (2 mg/kg/day) for 5 weeks and then administrated alpha-tocopherol (20 mg/kg/day) for the following 5 weeks; (4) EGb761-HAL group (EGb), rats were injected with HAL (2 mg/kg/day) for 5 weeks and then administrated EGb761 (50 mg/kg/day) for the following 5 weeks.