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Research report

Beneficial effects of EGb761 and vitamin E on haloperidol-induced vacuous chewing movements in rats: Possible involvement of S100B mechanisms

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HIGHLIGHTS

- To compare the effects EGb761 with that of vitamin E for TD and S100B expression in four brain regions.
- Both EGb761 and vitamin E significantly prevented the development of TD.
- Both EGb761 and vitamin E significantly reversed the increased S100B in all studied brain regions.
- Both EGb761 and vitamin E reduce TD symptoms, possibly via their effects on S100B signaling.

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ABSTRACT

Tardive dyskinesia (TD) is a serious side effect induced by the long-term administration of typical antipsychotics. The pathophysiology of TD remains unclear, but experimental evidence suggests that neurodegeneration caused by free radicals may play an important role in TD development. S100B is considered a potential biomarker of structural neural and glial damage. This study investigated S100B expression in TD-related brain regions and assessed the effect of antioxidants *Gingko biloba* leaf extract (EGb761) and vitamin E (VE) on S100B in TD rats. A total of 32 rats were randomly divided into 4 study groups: saline control (saline), haloperidol alone group (Hal), EGb761-haloperidol (EGb-Hal), and vitamin E-haloperidol (VE-Hal). Rats were treated with haloperidol intraperitoneal injections (2 mg/kg/day) each day for 5 weeks. EGb761 (50 mg/kg/day) and VE (20 mg/kg/day) were then administered during a 5-week withdrawal period. We performed behavioral assessments and immunohistochemically analyzed S100B expression in four TD-related brain regions. Our findings demonstrated that haloperidol administration led to a progressive increase in VCMs and in S100B expression in all four brain regions. Both EGb761 and VE reversed these changes, and there were no group differences between the EGb761 and VE groups. Our results indicated that long-term administration of haloperidol may induce VCMs and increase S100B expression in TD-related brain regions, and S100B may be a significant biomarker related to TD pathophysiology. Moreover, the antioxidant capacity of EGb761 and VE coupled with the possible neuroprotective effects of S100B may account for their success in improving the symptoms of haloperidol-induced TD.

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1. Introduction

Tardive dyskinesia (TD) is characterized by repetitive involuntary movements, usually involving the mouth, face, tongue, and sometimes limb and trunk musculature. It occurs in 20–50% of patients treated chronically with antipsychotics and can manifest months or years after the initiation of antipsychotic treatment and persist after drug withdrawal [1–3]. However, the etiology and pathophysiology of TD remain poorly understood, and no well-accepted treatments are currently available [4]. Recent evidence suggests that free radical-induced neurodegeneration may be related to TD onset and progression [5–10]. Chronic neuroleptic administration can enhance free radical generation and damage the antioxidant defense system, leading to oxidative stress and lipid peroxidation, which can induce potentially irreversible damage in motor regions of the brain (*e.g.*, basal ganglia) that may be the mechanism underlying TD [11,12].

S100B is a calcium-binding protein predominantly produced and secreted by astrocytes that exerts paracrine and autocrine effects on neurons and glial cells. It plays important roles in cell proliferation and differentiation, cellular energy metabolism, and cytoskeletal modification [13,14]. S100B exerts neuroprotective or neurodegenerative effects depending on its concentrations. Nanomolar concentrations of S100B appear to have neuroprotective effects, stimulating neurite and astrocyte outgrowth and enhancing neuron survival during development, whereas micromolar concentrations induce neurodegenerative or apoptotic effects; thus, it has been considered as a potential biomarker of structural brain damage [15–19]. Several studies have investigated the change of serum or plasma and cerebrospinal fluid (CSF) S100B levels in humans and animal models for various conditions associated with brain damage or neurodegeneration [19]. The results for schizophrenia showed that peripheral S100B was increased in medicated and unmedicated schizophrenia patients, and increased S100B levels were correlated with negative and motor deficit symptoms [14,20–25]. Our previous study reported that schizophrenia patients with TD had higher serum S100B levels than those without, and serum S100B levels were positively correlated with abnormal involuntary movement scale (AIMS) scores [26], suggesting that increased S100B may play an important role in TD pathophysiology.

A number of studies have reported that vitamin E (VE), a highly efficient lipid-soluble free radical scavenger, exerts beneficial effects on TD [27–33]. In addition, a standardized extract of dried *Ginkgo biloba* leaves (EGb761) prepared according to a special manufacturing process that yields 24% flavonoids and 6% terpenes with less than 5 ppm of ginkgolic acids [34], acts as an excellent antioxidant and efficient scavenger of various reactive oxygen species [35] and has neuroprotective effects on disorders such as Alzheimer's disease (AD) and Parkinson's disease [36]. Our previous studies showed that EGb761 enhanced the effectiveness of the antipsychotic drug haloperidol [37] and improved the TD symptoms of schizophrenia [38]. However, there is no report regarding a possible regulatory role of EGb761 on S100B.

We hypothesized that the ameliorative effect of EGb761 on TD is associated with its effects on S100B expression in different brain regions. However, to our knowledge, no published studies have investigated S100B expression in TD-involved brain regions or the possible mechanism by which EGb761 improves TD symptoms in an animal model through S100B. Therefore, this study investigated S100B expression in the prefrontal cortex, striatum, substantia nigra, and globus pallidum of haloperidol-induced TD rats and assessed the effect of EGb761 treatment in TD rats. Because previous studies have confirmed that VE exerts benefit effects in TD, it was used as positive control.

2. Methods and materials

2.1. Animals

We used male Sprague Dawley rats, weighing between 180 and 220 g, which were obtained from the Central Animal House of Beijing University of Chinese Medicine. The animals were housed under standard laboratory conditions and maintained on a normal light–dark cycle with 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.

2.2. Drugs

Haloperidol (HAL) (Shanghai Jiufu Pharmaceutical Co., Ltd., China) was diluted with normal saline (NS) to the concentration of 1 mg/ml, vitamin E (VE) (Shanghai Roche Pharmaceutical Co., Ltd., China) and *Ginkgo* leaf extract (EGb761) (Beijing University of Chinese Medicine, China) was diluted with NS to the appropriate concentration. All compounds were administered in a constant volume of 5 ml/kg of rat body weight.

2.3. VCM measurement

Three types of oral behavior were recorded: vertical jaw movements, bursts of jaw tremor, and tongue protrusions. For calculation purposes, each burst of purposeless chewing or jaw tremor was scored as 1 VCM if its duration was at least 3 s. A VCM was defined as vertical and rapid jaw movements in the absence of any chewable material in the rat's mouth and that did not appear to be caused by any particular stimulus. In addition, the count 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 VCMs gradually increased with HAL (2 mg/kg/day intraperitoneally) injection and reached a plateau in the 5th week [39]. Hence, in the present study, the animal model was established by injecting HAL (2 mg/kg/day, intraperitoneally) for 5 weeks.

2.4. Treatment schedule

A total of 32 rats were randomly divided into 4 groups ($n = 8$ each). (1) Saline control group (saline): rats were injected intraperitoneally with saline for 5 weeks, and then received saline by oral gavage for the following 5 weeks; (2) haloperidol alone group (haloperidol): rats were injected with HAL (2 mg/kg/day) for 5 weeks, and then administered saline for the following 5 weeks; (3) VE-haloperidol group (VE group): rats were injected with HAL (2 mg/kg/day) for 5 weeks, and then administered VE (20 mg/kg/day) for the following 5 weeks; (4) EGb761-haloperidol group (EGb761 group): rats were injected with HAL (2 mg/kg/day) for 5 weeks, and then administered with EGb761 (50 mg/kg/day) for the following 5 weeks.

2.5. Behavioral assessment

Behavioral assessment was carried out as previously described [10,40,41]. Briefly, rats were placed individually in a small 20 × 30 × 30-cm Plexiglas cage for VCM assessments. Animals were allowed 10 min to accommodate to the observation cage before behavioral assessment. The behavioral parameters of VCMs were continuously measured for a period of 5 min [42]. In all experi-