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Coadministration of hydroxysafflor yellow A with levodopa attenuates the dyskinesia

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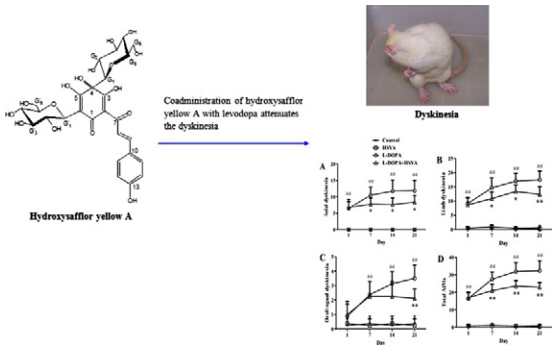
HIGHLIGHTS

- Coadministration of hydroxysafflor yellow A with levodopa
- Attenuates dyskinesia
- Regulating the expression of the dopamine D_3 receptor

GRAPHICAL ABSTRACT



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ABSTRACT

Levodopa (L-DOPA) is used as the most effective drug available for the symptomatic treatment of Parkinson's disease (PD). However, long-term treatment of L-DOPA frequently causes complications, including abnormal involuntary movements such as dyskinesia and response fluctuations in PD patients. In the present work, we investigated whether hydroxysafflor yellow A (HSYA) ameliorates L-DOPA-induced dyskinesia and motor fluctuations in the 6-hydroxydopamine-lesioned rat model of PD. Valid PD rats were treated daily with vehicle, HSYA alone, L-DOPA, or a combination of HSYA plus L-DOPA for 21 days, respectively. L-DOPA (8 mg/kg) and benserazide (15 mg/kg) were treated intraperitoneally. HSYA was administrated intraperitoneally at a dose of 10 mg/kg. The abnormal involuntary movements and rotational behavior were evaluated. The expression of the dopamine D_3 receptor in the striatum was also assayed. The results demonstrated that daily administration of L-DOPA to PD rats for 21 days induced a steady expression of dyskinesia. Coadministration of HSYA with L-DOPA significantly ameliorated L-DOPA-induced dyskinesia. The combination treatment also prevented the shortening of the motor response duration that defines wearing off motor fluctuations. HSYA also inhibited the increase of expression of the dopamine D_3 receptor in the striatum. These findings demonstrated that HSYA provided anti-dyskinetic relief against L-DOPA in a preclinical model of PD via regulating the expression of the dopamine D_3 receptor. The combination of L-DOPA and HSYA also reduced the likelihood of wearing off development, and may thus support the utility of such compounds for the improved treatment of PD.

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with the loss of dopamine neurons in the nigrostriatal pathway, which leads to progressive dopamine depletion in the striatum. The most efficient treatment strategy for PD is replacement of dopamine by an exogenous supplement of its precursor levodopa (L-DOPA). In spite of its efficiency, long-term use of L-DOPA is associated with serious side effects consisting of motor response fluctuation and the emergence of drug-induced involuntary movements, so called L-DOPA-induced dyskinesia (LID). Ultimately, up to 80% of patients developed dyskinesia within 5 years of treatment, and some of those had to terminate the therapy due to severe LID. The presence of LID is troublesome and limits utility of L-DOPA in patients. It also significantly worsens the quality of life of the patients [1].

Research continues in an effort to resolve L-DOPA-related pathologies. However, current treatment options for LID are limited. The dose of L-DOPA is still the most significant variable in the development of dyskinesia [2], and lowering the dose of L-DOPA is the best strategy for avoiding L-DOPA-induced adverse effects [3]. However, lowering the dose of L-DOPA alone is not an ideal approach. Although a too-low dose is safer, it is less effective at alleviating symptoms and can even lead to extraneous disability [4]. Thus, a novel treatment method allowing an effective L-DOPA treatment with a low dosage is highly important. Increasing evidence implicated that drug combination therapy is a valuable approach to attenuate L-DOPA-induced adverse effects. Co-administration of l-stepholidine (one of the active ingredients of the Chinese herb *Stephania intermedia*) with L-DOPA significantly ameliorated LID without compromising the therapeutic potency of L-DOPA [5]. Previous study showed that oral coadministration of L-DOPA and rimonabant significantly decreased abnormal involuntary movements and dystonia [6]. Acupuncture and L-DOPA combination therapy reduces the effective dose of L-DOPA and alleviates LID [7]. Marin and colleagues demonstrated that the combination of L-DOPA and entacapone reduced the likelihood of motor fluctuation development [8].

Traditional Chinese medicine has attracted increasing attention as a complementary therapeutic method to Western medicine [9]. Hydroxysafflor yellow A (HSYA) is the main chemical component of the safflower yellow pigments. It was demonstrated that HSYA could attenuate the neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice [10]. Previous findings in our laboratory also supported a role for HSYA in preserving dopamine neuron integrity and motor function in a rodent model of PD [11]. However, the possible prevention of L-DOPA-induced dyskinesia and wearing off with the coadministration of HSYA and L-DOPA is still unknown. In this study, we investigated whether the coadministration of HSYA and L-DOPA can prevent L-DOPA-induced dyskinesia and wearing off in 6-hydroxydopamine (6-OHDA)-induced PD in rats.

2. Methods

2.1. Animals

Male Sprague–Dawley rats weighing 220–240 g were acquired from the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs (Yantai, China). Animals were housed in a climate-controlled room, maintained on a 12 h/12 h light/dark cycle, and given food and water ad libitum. The experiments were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (publication 86-23, revised in 1986) and were approved by the Local Ethics Committee.

2.2. Drug and chemical agents

HSYA (98% purity by HPLC) was obtained from Shandong Luye Pharmaceutical Co., Ltd. (Yantai, China). HYSA was dissolved in normal saline and then administrated intraperitoneally to the rats. Apomorphine hydrochloride, L-DOPA, 6-hydroxydopamine (6-OHDA), desipramine hydrochloride, and ascorbate were obtained from Sigma Co. (St. Louis, MO, USA). An anti-dopamine D_3 receptor was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence (ECL) detection reagents and BCA protein assay kits were obtained from Beyotime Institute of Biotechnology (Haimen, China).

2.3. Unilateral 6-OHDA lesion

Surgery was conducted as previously described [5], with minor modifications. Briefly, the rats were anesthetized with sodium pentobarbital (30 mg/kg, *i.p.*) and placed in a stereotaxic frame with the incisor bar positioned 4.5 mm below the interaura line. Each animal received a 6-OHDA injection (16 μg of 6-OHDA in 4 μL of saline with 0.02% ascorbate) into the left medial forebrain bundle by means of a Harvard infusion pump (Harvard Apparatus Inc., Holliston, MA, USA). Stereotaxic injections were placed 4.0 mm anterior to the interaural line, 1.65 mm lateral to the midline, and 8.0 mm ventral to the surface of the skull. The injection rate was 0.5 μL/min, and the needle was kept in place for an additional 5.0 min before being slowly retracted. To limit the damage to adrenergic neurons, desipramine hydrochloride (25 mg/kg, *i.p.*) was administered 30 min before the 6-OHDA injections. Three weeks after surgery, apomorphine (0.1 mg/kg, *i.p.*) was administered to the rats. The rats with contralateral rotations of more than 150 rotations per 30 min were used as a valid PD model. According the rotations, the valid PD model rats were divided randomly into four groups: control group ($n = 8$, rotations $= 177.8 \pm 15.1$), HSYA group ($n = 8$, rotations $= 178.0 \pm 11.2$), L-DOPA group ($n = 10$, rotations $= 179.7 \pm 13.4$), and L-DOPA + HSYA group ($n = 10$, rotations $= 178.2 \pm 12.4$).

2.4. Drug treatments

The rats were administrated intraperitoneally with vehicle, or HSYA (10 mg/kg), or L-DOPA (8 mg/kg with 15 mg/kg benserazide), or L-DOPA (8 mg/kg with 15 mg/kg benserazide) plus HSYA (10 mg/kg) daily for 21 consecutive days. For L-DOPA plus HSYA treatment, rats received 10 mg/kg of HSYA 30 min prior to L-DOPA treatment. L-DOPA, benserazide, and HSYA were dissolved in normal saline. Each drug was administered in a final volume of 1 mL/kg body weight. Abnormal involuntary movements (AIMs) were measured on days 1, 7, 14 and 21 of treatment. Rotational behavior was observed on days 1 and 21. The dosage of 10 mg/kg HSYA was chosen based on our pilot study.

2.5. Abnormal involuntary movements

Rats were monitored for AIMs using a procedure and score system according to previous methods [12,13,14]. On test days, rats were individually placed in plastic trays (60 × 75 cm) at 5 min before treatments. After L-DOPA injection, rats were observed individually for 1 min every 20 min for a total of 240 min after injection of the drugs or vehicle. The experienced observer was kept blinded to animal grouping and treatment throughout the entire experiment. Each rat was scored for exhibition of the following three categories of AIMs: (1) axial, lateral flexion and axial rotation of the neck and trunk towards the side contralateral to the lesion; (2) limb, repetitive, rhythmic jerky movements or dystonic posturing of the forelimb on the side contralateral to the lesion; (3) orolingual, tongue protrusion without the presence of food or other objects. For each observation period of 1 min, a score of 0–4 was assigned for each category based on the following criteria: 0, absent; 1, present for less than half of the observation time; 2, present for more than half of the observation time; 3, present all the time but suppressible by threatening stimuli; 4, present all the time and not suppressible. The axial, limb and orolingual AIMs will be integrated as total AIMs scores per session. For assessing rotational behavior, each