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Protective effects of pinostilbene, a resveratrol methylated derivative, against 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y cells

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Abstract

Resveratrol (3,4′,5-*trans*-trihydroxystilbene) is a phytoalexin with emerging lines of evidence supporting its beneficial effects on cardiovascular systems and inhibition of carcinogenesis. It has also been reported that certain methylated resveratrol derivatives are more effective than resveratrol in the prevention/treatment of cancer. However, little is known about the impact of resveratrol and its derivatives on the development of Parkinson's disease. In this study, we compared the neuroprotective effects of resveratrol with four methylated (fully or partially) resveratrol derivatives against parkinsonian mimetic 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in SH-SY5Y cells. Release of lactate dehydrogenase and activity of caspase-3 triggered by 6-OHDA were significantly reduced by resveratrol and one of the methylated derivatives, pinostilbene (3,4′-dihydroxy-5-methoxystilbene), in a dose-dependent manner. In addition, pinostilbene exerted a potent neuroprotective effect with a wider effective concentration range than resveratrol. By using high-performance liquid chromatography, we found that uptake of pinostilbene into SH-SY5Y cells was significantly higher than that of resveratrol. Enhanced bioavailability may thus be a major factor contributing to the neuroprotective activity of pinostilbene. Moreover, Western blot analysis demonstrated that pinostilbene markedly attenuated the phosphorylation of JNK and c-Jun triggered by 6-OHDA. Besides, mammalian target of rapamycin kinase may be an intracellular target accounting for the neuroprotective effects of pinostilbene. Our findings demonstrate the potential of methylated stilbenes in neuroprotection and provide important information for further research in this field.

Keywords: Parkinson's disease; 6-Hydroxydopamine; Resveratrol; Pinostilbene; Neuroprotection

1. Introduction

Resveratrol (3,4′,5-*trans*-trihydroxystilbene) is a stilbene-type phytoalexin in plants such as grapes, peanuts, berries and pines [1]. It is produced in these plants to counteract environmental stresses such as UV irradiation and fungal infection. Among the wide range of biological and pharmacological activities, resveratrol has been intensively investigated as a cancer chemopreventive agent. It is reported to suppress the growth of several tumor cell lines such as leukemic, prostate, colonic, breast and esophageal cells through inhibiting tumor initiation, promotion and/or progression [1,2]. Resveratrol is also reported to be one of the active constituents of Itadori tea, which has been used as a traditional medicine mainly for curing heart disease and stroke in China and Japan [2]. There are considerable epidemiological reports pointing to an inverse association between moderate consumption of red wine and the incidence of coronary heart disease; resveratrol is widely regarded as one of the major phenolic compounds in red wine attributing to cardioprotection. Several mechanisms have been proposed to account for resveratrol's cardioprotective activities such as free-radical scavenging and the inhibition of cyclooxygenase and hydroperoxidase; the latter two result in attenuating platelet aggregation and reducing lipid peroxidation, respectively [1,3].

Studies on the neuroprotective activity of resveratrol have mainly focused on animal models with disorders or injury to the central nervous system such as Alzheimer's disease (AD),

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Parkinson's disease (PD), Huntington's disease, cerebral ischemia or traumatic brain injury [4,5]. Increasing lines of evidence suggest that acute or chronic treatment with resveratrol can confer protective effects on neurons against colchicine-, 3-nitropropionic-acid- or trauma-induced cognitive and motor impairment as well as hippocampal neuron loss [6–9]. The underlying mechanisms can be attributed to the ability of resveratrol to alleviate oxidative stress by reducing the elevated malondialdehyde, lipid peroxidation, nitric oxide and xanthine oxidase. On the other hand, it can restore the levels of glutathione and increasing succinate dehydrogenase activity in the brains from animal experiments. In addition, administration of resveratrol protects cerebral neurons from ischemia-induced damage [10] and 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine-induced motor coordination impairment, hydroxyl radical overloading and neuronal loss probably via scavenging of free radicals [11].

The usefulness of resveratrol, however, is limited by its instability upon exposure to light and oxygen or in environments with drastic pH conditions. These stimuli may cause *trans–cis* transformation or oxidation that leads to reduction in bioavailability and bioactivity [12]. An effective approach to stabilize resveratrol is accomplished by methylation of its hydroxyl groups to form trimethylated resveratrol. It has been demonstrated that the trimethylated resveratrol is more effective than resveratrol in preventing CCl_4-induced liver damage, via inhibiting lipid peroxidation and serum enzyme activity of γ-glutamyl transpeptidase. Trimethylated resveratrol has been postulated to act as a prodrug of resveratrol, which improves the bioavailability of resveratrol in target tissues [13]. Similarly, trimethylated resveratrol or 3,5-dimethoxy-4′-hydroxystilbene has been reported to exert up to 100-fold stronger cytotoxicity than resveratrol in cancer cell lines by depleting the intracellular pool of polyamines and by altering microtubule polymerization [14]. However, little is known about the relative efficacy of methylated resveratrol and resveratrol in terms of neuroprotection. This motivated us to synthesize partially and fully methylated resveratrol derivatives and investigate their protective effects on Parkinsonian mimetic 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. The synthesized compounds include 3,4′,5-trimethoxystilbene (R1), 3,4′-dimethoxy-5-hydroxystilbene (R2), 3,4′-dihydroxy-5-methoxystilbene (R3, pinostilbene) and 3,5-dihydroxy-4′-methoxystilbene (R4, desoxyrhapontigenin) (Fig. 1). Their neuroprotective activities were evaluated in dopaminergic human SH-SY5Y cells by monitoring their effects on the level of lactate dehydrogenase (LDH) release and the activity of caspase-3 triggered by 6-OHDA. Bioavailability and stability of resveratrol and the methylated derivatives were analyzed by high-performance liquid chromatography (HPLC). Furthermore, their effects on the inhibition of 6-OHDA-activated JNK pathway as well as the modulation of mammalian target of rapamycin (mTOR) kinase activity were also evaluated with Western blot analysis.

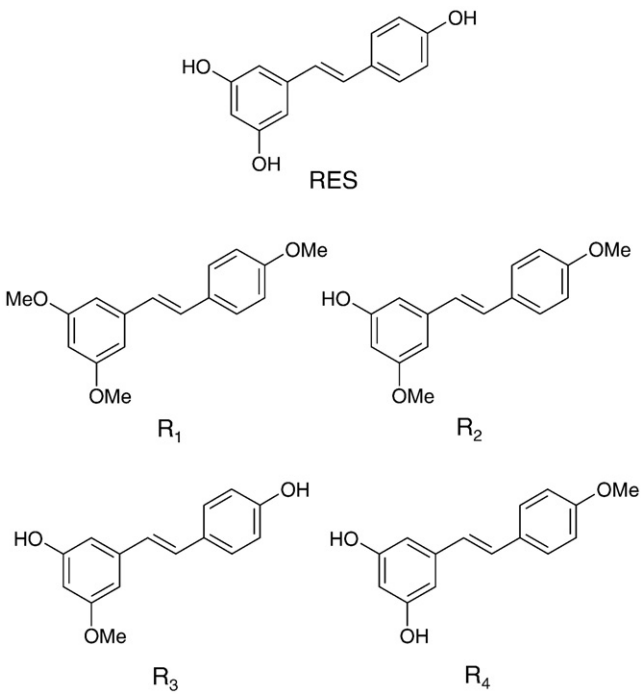

Fig. 1. Structures of resveratrol and synthesized methylated resveratrol derivatives.

2. Materials and methods

2.1. Materials

Materials used for SH-SY5Y cell cultures were purchased from Gibco-BRL (Burlington, Ontario, Canada). For organic synthesis of stilbenes, all starting materials with the highest quality were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were obtained from the following companies: resveratrol, 6-OHDA, 1,1-diphenyl-2-picrylhydrazyl (DPPH), protease inhibitor cocktail, phosphatase inhibitor cocktail and anti-β-actin monoclonal antibody were obtained from Sigma-Aldrich, Inc. Caspase-3 substrate (Ac-DEVE-pNA) was purchased from Calbiochem, Inc. (La Jolla, CA, USA). LDH cytotoxicity assay kit was obtained from Roche Diagnostics (Mannheim, Germany). HPLC analysis was performed on a Shimadzu LC-20AT system equipped with a diode array detector and LC-Solution software. Rabbit polyclonal anti-phosphorylated JNK (Thy183/Tyr185) antibody, rabbit monoclonal anti-phosphorylated c-Jun-I (Ser73) antibody, rabbit monoclonal anti-phosphorylated mTOR (Ser 2448) antibody and rabbit monoclonal anti-phosphorylated GSK-3β (Ser 9) antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). Horseradish-peroxidase-conjugated goat anti-rabbit and goat anti-mouse antibodies were obtained from DAKO (Glostrup, Denmark). PVDF membrane was from Bio-Rad (Richmond, CA, USA). Biomax X-ray film was from Kodak (Tokyo, Japan). Visualizer Spray & Glow ECL Western Blotting Detection System was from Millipore (Billerica, MA, USA).

2.2. Synthesis of methylated RES derivatives

Dry potassium carbonate (1.38 g, 10 mmol) was added to a solution of resveratrol (1.163 g, 5 mmol) in dry acetone (16.25 ml). Methyl iodide (0.472 ml, 7.5 mmol) was added dropwise. The reaction mixture was stirred for 24 h at room temperature and then poured onto 20 g of ice, and acetone was removed under reduced pressure by a rotary evaporator. The obtained aqueous phase was extracted with ethyl acetate (3×20 ml). The organic phase was dried with $MgSO_4$, and ethyl acetate was removed under reduced pressure. The crude residue from above was then subjected to flash chromatography on silica gel (chloroform/ethyl acetate, 7:1) to afford R1 (85.0 mg) and R2 (290.1 mg), a subfraction with two components and the starting resveratrol. The impure subfraction was further purified by gel-filtration chromatography on Sephadex LH-20 column (chloroform/methanol, 1:1) to offer R3 (154.5 mg) and R4 (363.9 mg). The structures of these stilbene compounds were confirmed by analysis and comparison of spectral data (NMR and MS) with literature [15].

2.3. Cell culture and treatment

Dopaminergic SH-SY5Y neuroblastoma cells (passage number ≤22) were cultured with MEM supplemented with 10% heat-inactivated FBS, L-glutamine (2 mM), penicillin (50 U/ml) and streptomycin (50 μg/ml) at 37°C in a humidified 5% CO_2 incubator. Stock solutions of methylated derivatives and RES (10 mM) were prepared in dimethyl sulfoxide (DMSO). All treatments were performed when cells were at ~80% confluence. Just before treatment, the culture medium was replaced with treatment medium [MEM supplemented with 3% FBS, 1% L-glutamine (2 mM), 1% penicillin (50 U/ml) and streptomycin (50 μg/ml)]. Different concentrations of stilbenes were diluted in the treatment medium. The vehicle DMSO alone (maximal concentration: 0.5 μl/ml treatment medium) had no influence on the growth of the cells. Cells pretreated with or without compounds for 30 min were exposed to 25 μM 6-OHDA.

2.4. LDH activity assay

When cells undergo necrosis, LDH can be released from inside cells with damaged membrane. To evaluate the general cytotoxicity, LDH activity assay was