Rhus verniciflua Stokes Attenuates Glutamate-induced Neurotoxicity in Primary Cultures of Rat Cortical Cells

Eun Ju Jeong1, Sang Hyun Sung1, Jinwoong Kim1, Seung Hyun Kim2, and Young Choong Kim1,*

1College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151-742, Korea
2Institute for Life Science, Elcom Science Co. Ltd., Seoul, Korea

Abstract – The methanolic extract of Rhus verniciflua Stokes (RVS-T) and its fractions (RVS-H, RVS-C, RVS-E and RVS-B) showed significant neuroprotective activity against glutamate-induced toxicity in primary cultures of rat cortical cells. RVS-B, which showed the most potent neuroprotective activity, was further fractionated to yield RVS-B5. Treatment of cortical cells with the RVS-T, RVS-B and RVS-B5 reduced the cellular ROS level and restored the reduced activities of glutathione reductase and SOD induced by glutamate. Although, the activity of glutathione peroxidase was not virtually changed by glutamate, RVS-B5 increased the glutathione peroxidase activity. In addition, these three tested fractions significantly restored the content of GSH which was decreased by glutamate insult in our cultures. Taken together, it could be postulated that RVS extract, in particular its fraction RVS-B5, protected neuronal cells against glutamate-induced neurotoxicity through acting on the antioxidative defense system.

Keywords – Rhus verniciflua Stokes, neuroprotection, primary cultures of rat cortical cells, glutamate, antioxidant activity

Abbreviations – ROS; reactive oxygen species, SOD; superoxide dismutase

Introduction

Glutamate is found naturally in millimolar levels in the brain and plays a dominant role in central excitatory neurotransmission. This transmission is involved in such activities as neuronal survival, synaptogenesis, neuronal plasticity, learning and memory processes (Albright et al., 2000). However, excessive amount of glutamate is also recognized as causing neuronal cell loss (Choi, 1988; Coyle and Puttfarcken, 1993). Abnormalities in glutamate neurotransmitter systems may be involved in neurological disorders such as seizures (Lipton and Rosenberg, 1994), ischemia and spinal cord trauma (Chase and Oh, 2000; Heintz and Zoghbi, 2000) and neurodegenerative disorders including Alzheimer’s disease (Choi and Rothman, 1990) and Parkinson’s disease (Lee et al., 1999). In view of this, neuroprotection against glutamate-induced neurotoxicity has been a therapeutic strategy for preventing and treating neurodegeneration (Muir and Lees, 1995; Trist, 2000; Meldrum, 2002).

In the course of searching natural product with protective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells, we found that an extract of the stem bark of Rhus verniciflua Stokes (RVS) exhibited significant neuroprotective activity. RVS has been traditionally used as an ingredient in Korean and Chinese medicines to treat gastritis, stomach cancer, arteriosclerosis (Kim, 1996). A variety of flavonoids such as fustin, fisetin, butein and sulfuretin have been isolated from this plant (Lee et al., 2002; Park et al., 2004). Recent studies have reported the antioxidant activity of RVS extract by scavenging reactive oxygen species (ROS) (Jung et al., 2006; Park et al., 2007). In addition, anti-apoptotic, anti-rheumatoid arthritis and antimutagenic activities of RVS were found to be mediated via antioxidant activity (Lim et al., 2000; Choi et al., 2003; Park et al., 2004).

Oxidative stress is well-known mechanism responsible for glutamate-induced neuronal degeneration (Coyle and Puttfarcken, 1993). Hence, we assessed effects of RVS extract and its fractions against toxicity induced by glutamate in primary cultures of rat cortical cells. To elucidate the mechanism, we assessed the effects of the extract and its fractions on cellular ROS level and the activities of glutathione peroxidase, glutathione reductase and superoxide dismutase (SOD), and the content of reduced glutathione (GSH) in glutamate-injured cells.

*Author for correspondence
Fax: +82-2-888-2933; E-mail: youngkim@snu.ac.kr
Experimental

Preparation of RVS samples – The stem bark of RVS was purchased from Kyungdong Oriental Herbal Market (Seoul, Korea). Dried stem bark of RVS (12 kg) was extracted with 80% MeOH in an ultrasonic apparatus. The methanolic extract (RVS-T) was concentrated in vacuo to give a crude extract (1.13 kg). This methanolic extract was suspended in H2O and fractionated successively with n-hexane, CHCl3, ethylacetate (EtOAc) and n-butanol (n-BuOH) to yield RVS-H (36 g), RVS-C (91.6 g), RVS-E (101.3 g) and RVS-B (102 g), respectively. RVS-B which showed the most potent neuroprotective activity was subjected to column chromatography on HP column eluting with a gradient of MeOH-water (0 : 100 → 1 : 4 → 2 : 3 → 3 : 2 → 4 : 1 → 100 : 0) to give six subfractions; RVS-B1 (66.6 g), RVS-B2 (9.5 g), RVS-B3 (8.2 g), RVS-B4 (7.2 g), RVS-B5 (6.5 g), RVS-B6 (1.4 g).

Chemicals – All chemicals for rat cortical cell cultures and biochemical assays were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, U.S.A.). Fetal bovine serum was obtained from Hyclone Co. (Logan, UT, U.S.A.).

Cell culture – Primary cultures of mixed cortical cells containing both neuronal and glial cells were prepared from 17 to 19-day-old fetal Sprague-Dawley rats as described previously (Kim et al., 1998). In brief, the trypsin-dissociated cortical cells were plated on multiwell culture plates (Corning, NY) coated with collagen at a density of 1 × 106 cells per well. The cortical cells were grown in DMEM containing 10% heat-inactivated FBS and streptomycin (100 µg/mL) at 37 °C in a humidified atmosphere of 95% air-5% CO2. All experiments were performed with Ethical Approval from the Seoul National University.

Neurotoxicity and cell viability – Samples for the test were dissolved in DMSO (final culture concentration, 0.1%); preliminary studies indicated that the solvent had no effect on cell viabilities of control cells and glutamate-treated cells at the concentration used (Kim et al., 1998). Seventeen-day-old cortical cell cultures were pretreated with the samples for 1 h and then exposed to 100 µM L-glutamate. After 24 h incubation, the cultures were assessed for neurotoxicity. Neuronal cell viability was quantified by MTT assay, which reflects mitochondrial succinate dehydrogenase function (Kim et al., 1998).

Measurement of cellular peroxide – The relative level of free radicals, that is peroxide, in cultured cells was measured with the oxidation-sensitive compound, 2,7-dichlorofluorescin diacetate (DCF-DA) by the method of Goodman and Mattson (1994). Cells were loaded with DCF-DA (50 µM, 50 min incubation) followed by three times washes in HBSS. DCF fluorescence was then determined after 3 h incubation by measuring light emitted at 530 nm of exciting cells with light at 485 nm. Values shown are the mean ± S.D.

Assay for the activities of antioxidant enzymes – Cells from three culture plates were pooled in 2 mL of 0.1 M phosphate buffer (pH 7.4) and homogenized. The homogenate was centrifuged for 30 min at 3000 g at 4°C and the supernatant was used for the measurements of antioxidant enzyme activity and GSH contents. The activity of SOD was determined according to the method of McCord and Fridovich (1969) by the xanthine-xanthine oxidase reaction. Glutathione reductase activity was measured according to the method of Carlberg and Mannervik (1975) based on the reduction of GSSG by glutathione reductase in the presence of NADPH. The activity of glutathione peroxidase was determined by quantifying the rate of oxidation of GSH to GSSG by cumene hydroperoxide (Hohe and Gunzler, 1984). Values shown are the mean ± S.D.

Determination of total GSH content – Total GSH in the supernatant was determined spectrophotometrically using the enzymatic cycling method (Tietz, 1969). Values shown are the mean ± S.D.

Determination of protein concentrations – Protein concentrations were determined using a bicinchoninic acid (BCA) kit (Sigma, MO, USA) with bovine serum albumin as a standard (Smith et al., 1985).

Statistical analysis – The levels of cell viability and antioxidant values were expressed as the mean ± S.D. and analyzed by one-way ANOVA. The data was considered to be statistically significant if the probability had a value of 0.05 or less.

Results

The effect of RVS on the neurotoxicity induced by glutamate – Neuroprotective activities of the RVS extract and its fractions were quantified by MTT assay in primary cultures of rat cortical cells (Table 1). Under phase-contrast microscope, over 50% of neurons died in cortical cultures after exposure to 100 µM glutamate for 24 h. At a concentration of 100 µg/mL, all of the RVS fractions (RVS-T, RVS-H, RVS-C, RVS-E, RVS-B) significantly increased the viability of glutamate-treated cells. Among the fractions tested, RVS-B showed the most potent neuroprotective activity against glutamate-induced damage. Increment of the concentrations of the treated fractions over 100 µg/mL did not improve their