Oriental Pharmacy and Experimental Medicine 2001 **1(1)**, 75-78

Effect of Rhizoma Gastrodiae on cultured mouse spinal motor neurons damaged by xanthine oxidase and hypoxanthine

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SUMMARY

To evaluate the cytotoxic effect of oxygen radicals, xanthine oxidase(XO)/hypoxanthine(HX)-induced neurotoxicity was examined in cultured newborn mouse spinal motor neurons after spinal motor neurons were grown in the media containing various concentrations of XO/HX. And also, the protective effect of Rhizoma gastrodiae (RG) extract against XO/HX-induced neurotoxicity was evaluated. Cytotoxicity was measured as a cell viability adopted by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay. In this study, exposure of motor neurons to XO/HX induced cell death significantly, in a dose- and time-dependent manners in spinal motor neuron cultures. The decrease in cell viability of motor neurons damaged by XO/HX was proventioned by RG extract. These results suggest that the neuroprotective effect of RG extract on XO/HX-induced neurotoxicity may result from a attenuation of oxygen radicals.

Key words: Rhizoma gastrodiae, Xanthine oxidase, Spinal motor neurons

INTRODUCTION

Oxygen free radicals play a key role in multiful sclerosis as well as Aizheimer disease. They induce oxidative stress by oxygen free radicals (Difazio *et al.,* 1992). It has been suggested that a cumulation of oxygen free radicals and an injury of antioxidants result in considered important pathophysiological events in many a neurological disease (Lundgren *et al.,* 1991). Nowadays, the oxidative stress of oxygen free radicals is regarded as a causative factor in neurological diseases of the nervous system (Hall and Braughler, 1986; Rice-Evans and Diplock, 1993). oxygen free radicals, xanthine or hydrogen peroxide damages neurons by oxidant production, and results from the cell injury or cell death. Many researches have suggested that oxygen free radicals activate the lipid peroxidation chains and the damage of neuronal cells (Dykens *et al.,* 1987 and Harken *et al.,* 1988). Among of them, glucose oxidase causes breakdown of ATP following to contribution of xanthine, or glucose. A recent study reported that the mutation of the superoxide dismutase (SOD)-1 gene induces variant of familial amyotrophic lateral sclerosis (ALS). This gene mutation brings about the accumulation of superoxide radicals in brain and leads to neuronal injury. Therefore, oxygen free radicals are a casative factor in various neurological disease. Recently, it has been demonstrated that oxygen free radicals secrete excitotatory amino acids (EAAs) and they activate N-methyl-D-aspartate (NMDA) receptor leading to intra-cellular calcium influx (Pellegrini-Giampietro *et al.,* 1988; Lundgren *et al.,* 1991).

Recently, it has been demostrated that traditional oriental herbal medicinal prescriptions are very

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useful for the treatment of many neurological diseases. But, it has been left unknown on their preventional mechanism in neurological disease with oxygen radical-mediated neurological diseases (Dexter *et al.,* 1989).

In the present study, we measured cell injury induced by xanthine oxidase (XO) and hypoxanthine (HX) in cultured mouse spinal motor neurons. In addition, protective effects of Rhizoma Gastrodiae against XO / HX-induced neurotoxicity were examined.

MATERIALS AND METHODS

Cell culture

Spinal motor neurons derived from mouse were grown in 96 multiwell plates as described previously (Michikawa *et al.,* 1994). Spinal motor neurons were prepared by enzymatical dissociation from newborn mice. Dissociated spinal motor neurons were washed three times with Dulbecco's phosphate-buffered saline (PBS), and centrifugated at 80×g. The single cells were harvsted and divided in 96 multiwells coated with poly-L-lysine. Cells were plated as a density of 1×10^5 cells / well, and cells were incubated in 5% CO_2 / 95% air atmosphere at 37°C. Cells were used for these experiments after 5-10 days in culture.

Chemicals

Xanthine oxidase (XO) and hypoxanthine (HX) were purchased from Sigma Chemicals (St. Louis, Mo).

Preparation of Rhizoma gastrodiae (RG)

An extract of RG was prepared by dissolving the dried powder of herbs with distilled water. The extract was filtered, and then stored at 4°C before use. This materials was obtained from College of Oriental Medicine, Wonkwang University.

Exposure to XO and HX

Spinal motor neurons derived from newborn mouse were washed three times with PBS, and incubated with the media containing various concentrations of Rhizoma gastrodiae extract for 2 h at 37°C, 5% CO_2 / 95% air. After the incubation, cells were washed and treated with 5~40 mU / ml XO and 0.1 M HX for 1~6 h respectively, and processed for MTT assay.

MTT cytotoxicity assay

MTT assay was performed by the method of Mosman *et al. (*1983). After appropriate incubation periods of cells for the determination of cytotoxicity, final concentration of MTT stock solution (5 mg / ml) was added to each well, and incubated for 4 h at 37°C, 5% CO_2 / 95% air. After incubation, 96-well plates with cultures were measured on a Dynatech Microelisa reader at a wavelength of 570 nm.

Statistical analysis

Data was expressed as mean±S.D. The Student's *t*-test was used to significant with a *p*-value of less than 0.05.

RESULTS

Cytotoxicity of xanthine oxidase

To measure the dose-reponse relationship of XO-induced neurotoxicity on the cultured mouse spinal motor neurons, cells in 96 multiwells were exposed to concentrations of 5, 10, 20 and 40 mU / ml XO for 4 hours, and then processed for the MTT assay. At 5 mU / ml of XO, the number of living cells was about 78% of all the unexposed cells. At 10 mU / ml of XO, 63% of total cell popuration survived of XO-induced cytotoxicity. XO at a concentration of 20 mU / ml was 48% in cell viability after 4 h of exposure. At 40 mU / ml of XO, cell survival was reduced to 24% of the control (Fig. 1).

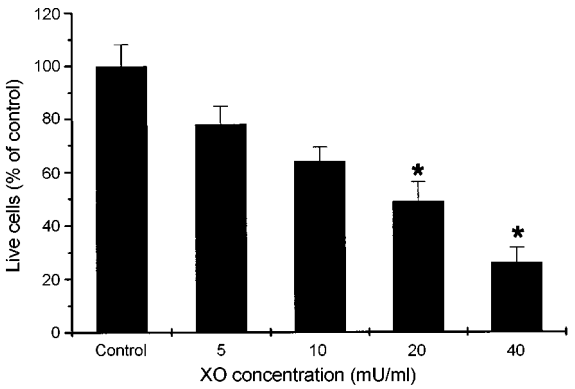

Fig. 1. A dose-dependency of xanthine oxidase (XO). XO-induced neurotoxicity was measured by MTT assay in cultured mouse spinal motor neurons. Cultures were exposed to 5, 10, 20 and 40 mU / ml XO for 4 h, respectively. The results indicate the mean ±SEM for 6 experiments. Significant difference from the control are marked with asterisks (*p<0.05).

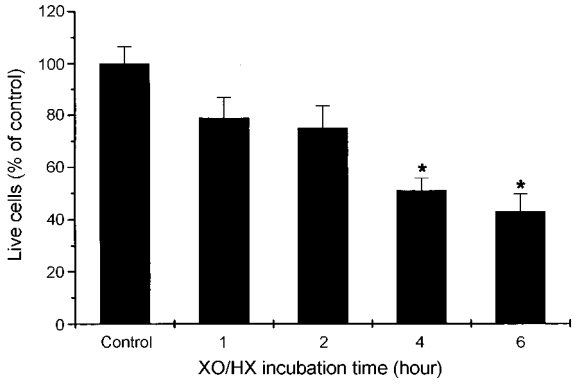

Fig. 2. A dose-dependency of xanthine oxidase (XO). XO-induced neurotoxicity was measured by MTT assay in cultured mouse spinal motor neurons. Cultures were exposed to 20 mU / ml XO / HX for 1, 2, 4 and 6 h, respectively. The results indicate the mean ±SEM for 6 experiments. Significant difference from the control are marked with asterisks (*p<0.05).

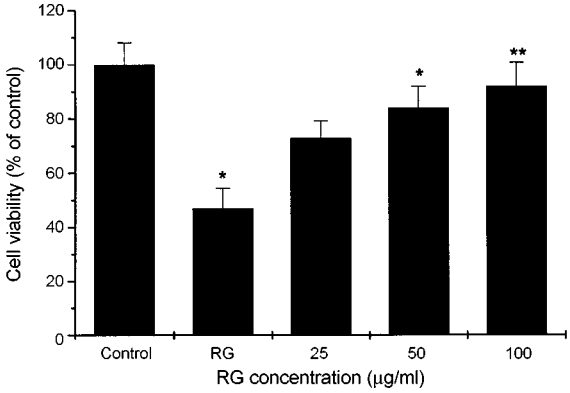

Fig. 3. Dose-response relationship of Rhizoma gastrodiae (RG) for its neuroprotective effect on XO / HX-induced neurotoxicity by MTT assay in cultured mouse spinal motor neurons. Cultures were preincubated with RG for 2 h before exposed to XO / HX. The results indicate the mean±SEM for 6 experiments. Significant difference from the control are marked with asterisks (*p<0.05, **p<0.01).

The effects of 20 mU / mlXO / 0.1 mMHX incubation time on cell survival are shown in Fig. 2. The cell viability was 77% after incubation of 1 h of exposure to XO / HX, and 72% after 2 h of exposure to XO / HX. After incubation of 4 h of exposure to XO / HX, cell survival was reduced to 51% of the control, and after incubation of 6 h of exposure to XO / HX, cell survival was reduced to 42% of the control (Fig. 2).

The effects of Rhizoma Gastrodiae (RG) extract on XO/HX induced cytotoxicity

Protective effect of RG extract tested for its ability to protect against XO / HX-induced cytotoxicity in the cultured mouse spinal motor neuron cultures. In this study, cultures were incubated in the media containing various concentrations of RG extract for 2 hours, and then cultures were exposed to 20 mU / ml XO / 0.1 mM HX for 4 hours. Cultures were processed for MTT assay. At 20 mU / ml XO / 0.1 mM HX alone for 4 h caused cell death in 48% of cell populations (Fig. 3). RG extract, herbal medicine extract, showed significant protection against XO / HX-induced neurotoxicity in cultured mouse spinal motor neurons. At 25 μg / ml of RG extract, the cell viability was 72% of the control. At 50 μg / ml of RG extract, cell survival was increased to 83% of the control At a 100 μg / ml concentration of RG extract, the cell survival rate was 88% in spinal motor neuron cultures (Fig. 3).

DISCUSSION

The toxic effect of xanthine oxidase (XO) / hypoxanthine (HX)-induced oxygen radical neurotoxicity on cultured spinal motor neurons was examined by MTT assay (Mosmann, 1983) and evaluated the potential protective effect of herbal extract of oriental medicine against XO / HX-induced neurotoxicity (Francois and Lang, 1886). In the present study, the cultured spinal motor neurons treated with XO / HX resulted in decreasing the survival of spinal motor neurons. From this result, it has suggested that XO / HX has neurotoxic effect on cultured mouse spinal motor neurons (Kim and Kim, 1991), and XO / HX-mediated oxygen radicals resulted in directly killing the cultured spinal motor neurons (Rubin and Faber, 1993). Therefore, oxygen radicals were regarded as a mediator of cell damage (Kim and Kim, 1991; Michikawa *et al.*, 1994). Also, we observed the protective effect in herbal extract of oriental medicine on XO / HX-induced neurotoxicity when Rhizoma gastrodiae (RG) extract was added 2 h before treatment of XO / HX in these experiments. In this study, the cell viability decreased by XO / HX was significantly increased by RG extract. From these results, it suggests that RG extract, which was taken up by neurons during the preincubation period before exposure to 20 mU / ml XO / 0.1 mM HX for 4 h, protected neurons from the XO / HX-

induced neurotoxicity. The mechanism of protective effect of RG extract against XO/HX-induced neurotoxicity is not clear at present, but it may be postulated that the protective effect of herbal extract is related with scavenging oxygen radicals. However, it must be confirmed by further studies biochemically or physiologically. From these results, we coinclude that XO/HX induced lethal toxic effect on cultured mouse spinal motor neurons and also, it is implied that herbal extract such as RG is effective in blocking XO/HX-induced neurotoxicity.

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