EFFECTS OF RHYNCHOPHYLLINE ON RAT CORTICAL NEURONS STRESSED BY METHAMPHETAMINE

Zhi Xian Mo[1]*, Dan Dan Xu[1], Ken Kin Lam Yung[2]

[1] School of Chinese Medicine, Southern Medical University, Guangzhou, China
[2] Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China
*Corresponding author: E-mail: cherrymo@fimmu.com

Summary

Objective: To reveal the neurotoxicity of Methamphetamine (MA) on cortical neurons in rats and to study the protective effects of Rhynchophylline (Rhy) on MA treated neurons. **Methods:** The neurotoxicity of MA was measured with MTT assay in primary cultured cortical neurons. The viability of neurons treated MA for 48 h combined with Rhy or ketamine was also tested. **Results:** MTT assay showed that MA had a nurotoxicity on rat cortical neurons and Rhy could protect against MA in culture viability. **Conclusion:** Rhy shows neuroprotective effect against MA in vitro. This property may also contribute to the neural activity of the origin of *Uncaria* species plants. These results suggest rhynchophylline may benefit for the treatment of MA exposure.

Key words: methamphetamine; rhynchophylline; ketamine; psychologic dependence; MTT assay

Methamphetamine (MA) is a abused psychostimulant. Repeated drug administration produces many changes of the brain functions. N-methyl-D-aspartate (NMDA) receptor, a kind of ionotropic glutamate receptor, is involved in mediating biological actions of the drug abuse, especially the psychostimulants (1). It was reported that MA appears to have a toxic effect on the neurons with a long-time exposure (2). In the present study, the neurotoxicity of MA was examined in cortical neurons which were abundant with NMDA receptors. We tried to find whether the NMDA receptor antagonist could attenuate the neurotoxicity of MA in vitro.

Rhynchophylline is the major tetracyclic oxindole alkaloid isolated from *Uncaria* species such as *Uncaria rhyncho- phylla* (Miq.) Jackson, *Uncaria macrophylla* Wall, and *Uncaria sinensis* (Oliv.). Gambirplant (Gouteng), as a traditional Chinese medicine, usually is used in the prescription mainly to treat ailments in the cardiovascular and central nervous systems, such as lightheadedness, con- vulsions, numbness, and hypertension, etc (3). The Rhyn- chophylline was the important active component in the *Uncaria rhynchophylla* and protected against glutamate- induced neuronal death in the rat cerebellar granule cells (4). Rhynchophylline was considered to act as noncompetitive antagonists of the NMDA receptor and that this property may contribute to the neuroprotective (5). Therefore, we invest- tigated the effects of rhynchophylline on methamphetamine- induced neurotoxicity, compared with a well known NMDA noncompetitive antagonist ketamine in the cortical neurons.

Materials and methods

Preparation of chemicals: Rhynchophylline (Rhy; purity: 99.7%; Matsuura Ykugyo CO., LTD. Japan) was dissolved in dimethyl sulphoxide (DMSO; Sigma, USA), then diluted in the sterile culture medium or PBS solution. The final concentration of the carrier (DMSO) was in less than 0.1%. Methamphetamine hydrochloride (National Laboratory for Narcotic Drugs, China) and ketamine hydrochloride (Jiangsu Hengrui Medicine Co., Ltd., P.R. China) were dissolved in sterile 0.9% NaCl solution directly.

Primary cell culture: Primary cell cultures of the cortical neurons were performed in accordance with the previous studies in our laboratory with slight modifications (6). In the present study, neonatal Wistar rats (postnatal day 1) were used. The rats were sacrificed by decapitation and brain regions of the cortex were dissected on ice. The brain tissues were put into an eppendorf with 1ml Minimal Essential Medium (MEM; Gibco, USA) with 1% penicillin- Streptomycin (PSN; Gibco, USA) in ice. The tissues were chopped using a razor blade in MEM. The chopped tissues were then incubated in a trypsin-EDTA solution with 1% PSN and 0.1% B27 supplement (Gibco, USA) at 37 °C in the 5% CO_2 incubator for 20 min, and agitated with a dropper every 5 min. MEM solution with 10% fetal bovine serum (FBS; Gibco, USA) and 1% PSN was added to stop the trypsinization. Then the solution was centrifuged at 1300 rpm for 10 min. The supernate was removed and the cells at the bottom were suspended in MEM solution and centrifuged again. Finally, the supernate was removed and the cells inside was resuspended in the plant medium (Neurobasal-A- Medium, 10% Fetal bovine serum, 1% PSN, 0.1%B27 supp- lement; Gibco, USA).

The neurons were then plated on poly-L-lysine (Sigma, USA) coated 96-well microplates. The cultures were maintained at 37 °C in the 5% CO_2 incubator. The maintain medium (Neurobasal-A-Medium, 1% PSN, 0.1%B27 supplement; Gibco, USA) with cytosine arabino- side (final concentration: 10^{-6} M; Sigma, USA) was used to exchange half of the previous plant medium every two days in order to inhibit the growth of glial cells.

MTT assay: Cell viability was determined by measuring the mitochondrial dehydrogenase activity (MDHA) using MTT assay. The cultured cortical neurons were used on day 6 in the present study. The cells were treated with MA at different concentrations, 100 μM ketamine or 100 μM Rhy, respectively, at 37 °C in the 5% CO_2 incubator for 48 h to determine effects of MA, ketamine and Rhy on normal neurons. In the drug-combined study, Ketamine or Rhy was added in culture medium10 min prior to 50μM MA to observed neuroprotective action of Ketamine or Rhy on neuronal damage induced by MA. Each test was performed triplicately in the 96-well microplates in which each well were plated with 1×10^5 neurons. After 48 h treatment with MA, 10 μl MTT solution (1 g/L; Sigma, USA) was added into each well, and the plate was incubated for 4 h. Formazan crystals, produced by the mitochondrial dehydrogenase in viable neurons, were dissolved by addition of 150 μl DMSO on the shaker at room temperature. The Absorbance of each well was measured using a microplate reader at 570 nm.

Statistical analysis: One-way analysis of variance (ANOVA) was applied to the comparison between groups. The inde- pendent-samples *t-test* was used in each case. The values are expressed as mean±SD. The significance level was computed at $P<0.05$ and $P<0.01$.

Results

Cell viability measurement for MA neurotoxicity: The changes in morphology of the cortical neurons were observed under the inverted microscope. The control neurons without MA treatment had large cell bodies with obvious dendrites and axons, which were used to communicate with the other neurons. The neurotoxic effect of MA was apparently observed in MA-treated neurons. After 48h MA exposure, the neurites of some neurons were disappeared. The cell bodies of partial neurons became round and some were floating (see Figure 1).

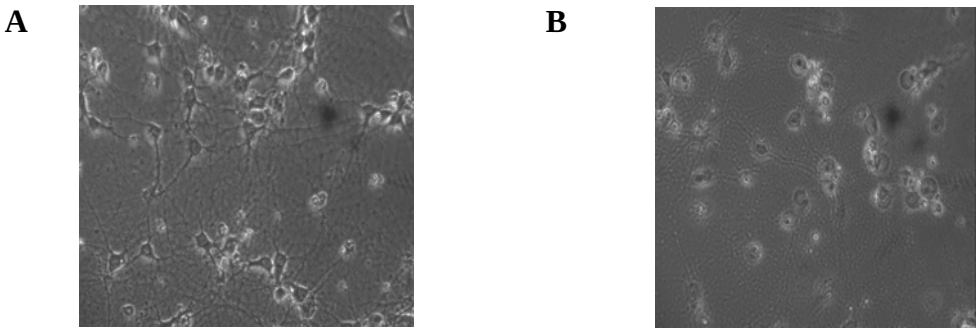

Figure 1. Phase-contrast photomicrographs of different treatment in cultures for 48 h. **A:** overview photograph of control neurons without MA treatment (magnification 200×); **B:** overview photograph of neurons treated with 150 μM MA (magnification 200×).

The neurotoxic effect of MA was measured with MTT cell viability test. The absorbance values at 570 nm represented the viable neuron numbers. As shown in the Figure 2, at the concentration range of 10-150 μM, MA showed a dose-dependent neurotoxicity. The absorbance in neurons treated with 50, 100, 150 μM MA for 48 h was significantly decreased compared with normal neurons ($P<0.05$).

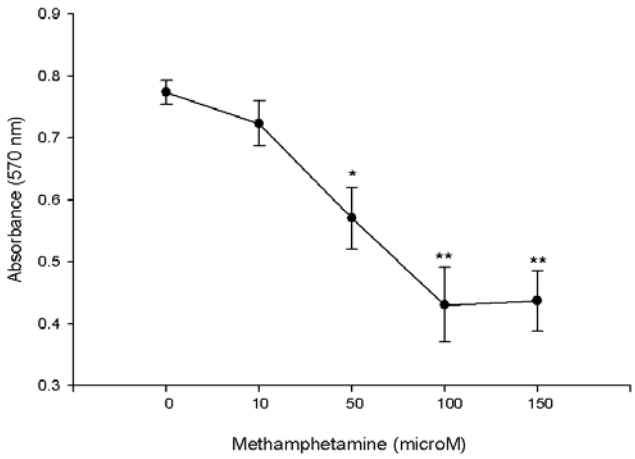

Figure 2 Neurotoxicity of methamphetamine measured by MTT assay. The cortical neurons were treated with various concentrations of MA (0, 10, 50, 100, 150 μM) triplicately for 48 h. The circle symbols represent the mean absorbances±S.E.M. at 570 nm. *P 0.05; **P 0.01 *vs* control neurons with 0 μM MA treatment.

Protective effect of rhynchophylline on the MA treated neurons: As shown in Figure 3, 100 μM ketamine and rhynchophylline did not show neurotoxic effects on the cortical neurons. Addition of 100 μM ketamine 10 min prior to 50 μM MA treatment did not prevent the cell death. The combination of ketamine and MA showed a significantly difference in comparison with normal neurons. Rhynchophylline appeared to have neuroprotective effect against MA in the cortical neurons. The protection of Rhy against MA appeared at the higher concentration (100 μM), but not the lower concentration (50 μM).

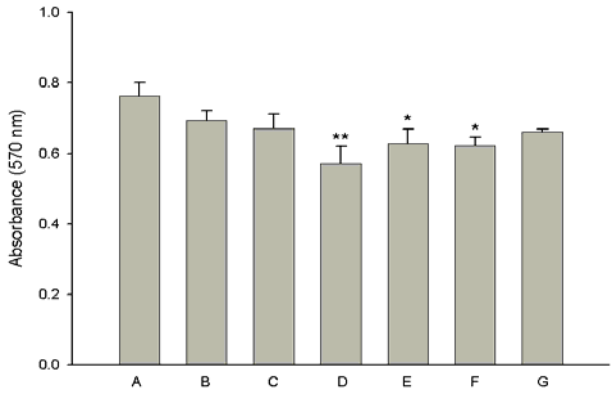

Figure 3 The effect of rhynchophylline, compared with ketamine, on the MA treated neurons in MTT assays. A: control: normal neurons, B: ketamine 100 μM. C: Rhy 100 μM. D: MA 50 μM. E: MA 50μM+ketamine 100μM. F: MA 50μM+Rhy 50μM. G: MA 50μM+Rhy 100μM. Each bar represents the mean absorbance±S.E.M. * P<0.05; ** P<0.01, *vs* control group.

Discussion

It is well known that MA induces the drug dependence by inhibiting dopamine transports (DAT) in dopaminergic neurons to increase the extracellular DA and indirectly affecting N-methyl-D-aspartate (NMDA) receptor partly in the system of reward pathways. In this study the cell viability of cortical neurons in vitro was measured with the treatment of MA at different concentration (0-150 μM) for 48 h. The neurotoxicity of MA was shown in a dose-dependent manner within the concentration range of 0-100 μM. When the concentration of MA was as high as 100 μM in the culture, the neurons were killed mostly. The neurotoxicity of MA was strong on cortical neurons in rats. Noncompetitive antagonists of the NMDA receptor ketamine was reported to provide some degree of neuronal protection against ischemia in the previous

study (7). But in this study, ketamine (100 μM) could not attenuate the neurotoxicity of MA (50 μM). Rhy appeared a neuroprotective action on rat cortical neurons at high concentration(100 μM). These results also indicate that the neurotoxicity of MA is not easy to be reversed indeed. Addition of Rhy prior to MA could protect the neurons.

In conclusion, our studies demonstrate that Rhy can protect the cortical neurons against MA exposure. This is the first report demonstrating an inhibitory effect of Rhy on MA impairment in vitro. These results suggest rhynchophylline is likely to benefit for the prevention or treatment of MA dependence to some extent. But more evidence in vivo and in vitro should be further provided.

Acknowledgments

This study was supported by the National Science Foundation of China, No.30371773.

References

1. Jones EA, Wang JQ, Mayer DC, McGinty JF. The role of dorsal striatal GABA(A) receptors in dopamine agonist- induced behavior and neuropeptide gene expression. Brain Res 1999; 836: 99-109.

2. Davidson C, Gow AJ, Lee TH, et al. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. Brain Res Rev 2001; 36:1-22.

3. Shi JS, Yu JX, Chen XP, et al. Pharmacological actions of Uncaria alkaloids, rhynchophylline and isorhynchophylline. Acta Pharmacol Sin 2003; 24:97-101.

4. Shimada Y, Goto H, Itoh T, et al. Evaluation of the protective effects of alkaloids isolated from the hooks and stems of Uncaria sinensis on glutamate-induced neuronal death in cultured cerebellar granule cells from rats. J Pharm Pharmacol 1999; 51:715-722.

5. Kang TH, Murakami Y, Matsumoto K, et al. Rhynchophylline and isorhynchophylline inhibit NMDA receptors expressed in Xenopus oocytes. Eur J Pharmacol 2002; 455:27-34.

6. Lui PW, Suen KC, Chan YS, et al. Striatal neurons but not nigral dopaminergic neurons in neonatal primary cell culture express endogenous functional N-methyl-D-aspartate receptors. Brain Res Mol Brain Res 2003; 120:9-21.

7. Basagan-Mogol E, Buyukuysal RL, Korfali G. Effects of ketamine and thiopental on ischemia reoxygenation-induced LDH leakage and amino acid release from rat striatal slices. J Neurosurg Anesthesiol 2005; 17:20-26.