

MASTER'S THESIS

Neuroprotective roles of ceftriaxone on cultured astrocytes and neuronal cells

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**Neuroprotective Roles of Ceftriaxone on Cultured Astrocytes and
Neuronal cells**

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Abstract

Glutamate is the neurotransmitter in the nervous system which can lead to neuronal damage and cell death when excess levels of glutamate accumulated. GLT-1 is a glial glutamate transporter that is found in glial cells. GLT-1 can rapidly remove glutamate from the extracellular space and can prevent glutamate toxicity. Beta-lactam antibiotics including ceftriaxone are potent stimulators of GLT1 expression. In order to investigate the efficacy of ceftriaxone in inducing the expression of GLT-1 in astrocytes, ceftriaxone was applied on primary cell culture of astrocytes. Different concentrations of the ceftriaxone (10, 100 μ M & 1 mM) were employed for 1 to 7 days. Expression of GLT-1 levels was quantified by immunofluorescence and Western blot experiments. The present results indicated that the levels of GLT-1 expression in the astrocytes were not changed with the increased concentrations of ceftriaxone by both Immunocytochemistry and Western Blotting. Ceftriaxone cannot stimulate the up-regulation of GLT-1 in cultured rat astrocytes.

Glutamate cytotoxicity assay was tested in astrocytes, SH-SY5Y cells and co-culture of astrocytes and SH-SY5Y cells to study the glutamate excitotoxicity effect. All the cells tested were sensitive to glutamate excitotoxicity. The cytotoxicity of SH-SY5Y cells in co-culture was reduced to the level similar to astrocytes culture. Astrocytes provide neuroprotection against glutamate toxicity to neurons in the co-culture system. The neuroprotective effect of ceftriaxone in pre-treated astrocytes against glutamate excitotoxicity was investigated. 100 μ M and 1mM of ceftriaxone showed a significant reduction of cytotoxicity in cultured astrocytes from 1 to 7 days of treatment. The neuroprotective effect of ceftriaxone in pre-treated astrocytes to SH-SY5Y cells against glutamate excitotoxicity was examined by co-culture. 100 μ M and 1mM of ceftriaxone also showed a significant reduction of cytotoxicity in co-culture. The cytotoxicity of co-culture after glutamate challenge has a larger reduction than astrocytes culture. Astrocytes showed a greater ability to protect SH-SY5Y cells against glutamate toxicity. Therefore, ceftriaxone is an effective drug against neuronal diseases.

NF- κ B is a possible mechanism for ceftriaxone to stimulate the GLT-1 expression. Ceftriaxone activates the GLT-1 expression at transcriptional level. By Western Blotting, *p*-NF- κ B p65 (Ser536) and NF- κ B p65 were not significant altered in cytoplasmic portion and absent in nuclear portion after ceftriaxone treatment. No significant change in *p*-I κ B α (Ser32) and I κ B α were found. These results revealed no activation of NF- κ B signaling pathway to induce GLT-1 transcription. Therefore, the

inactivation of NF- κ B signaling pathway revealed no change in GLT-1 expression after ceftriaxone stimulation. Akt play a critical role in controlling cell survival and apoptosis. Increase in *p*-Akt (Thr308) after 1mM of ceftriaxone treatment was found. This results revealed ceftriaxone provide neuroprotection by activating Akt signaling pathway. However, there is no significant change in *p*-Akt (Ser473), *p*-PDK1 (Ser241), *p*-PTEN (Ser380) and PI3K p110 γ . Other phosphorylation site of PDK1, PTEN and PI3K subunits should be performed to investigate the upstream target of Akt from ceftriaxone.

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