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GLT1 Glutamate Transporter Upregulation by Ceftriaxone Can Increase Glutamine Synthetase Expression In Acute Phase of Epileptogenesis

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Background and Aim : Epilepsy is defined as spontaneous recurrent seizures and is accompanied by many cellular and molecular abnormalities including gene expression and cellular reorganization. It is well described that one of the main mechanisms underlying these changes is glutamate excitotoxicity. Astrocytes play an important role in the epileptogenesis process as the main glutamate scavengers. Excess glutamate can be cleared by GLT-1 glutamate transporters and can be converted to Glutamine by Glutamine Synthetase (GS) enzyme. The glutamine is then transported to the nearby neurons for synthesis of GABA and glutamate. In this study, the effect of GLT-1 pharmacological upregulation on the expression of GS was assessed.

Methods : Male Wistar rats (200-280 g) were randomly divided into 4 groups (N=5): 1. Control group (received vehicle); 2. Pilocarpine group (temporal lobe epilepsy was induced using pilocarpine); 3. Pil+Cef group (animals received pilocarpine and 5 injections of Ceftriaxone 200 mg/kg); 4. Ceftriaxone group (receiving only 5 injections of Ceftriaxone 200mg/kg). Animal model was induced by an injection of lithium (127mg/kg) followed by pilocarpine i.p. administration (30mg/kg), 20 hours later. Ceftriaxone was injected 48 hours and 24 hours before and after pilocarpine (five consecutive days). Seizure behavior was monitored 4 hours and 3 weeks after model induction and animals showing spontaneous recurrent seizures were chosen for gene expression analysis, 72 hours and 31 days after model induction. To measure gene expression, RNA was extracted by conventional TRIzol method (RNX-Plus, SinaClone, Iran) from the hippocampus tissue. The concentration and integrity of extracted RNA was determined by UV spectrophotometry and gel electrophoresis. cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA). The primers were designed and synthesized by Macrogen, Inc. (Seoul, South Korea). Reaction system was 2X SYBR Green PCR Master mix (Parstous, Iran) 12.5 µl + upstream and downstream primers (10 pmol/ul) 1 µl each + cDNA template 1 µl, adding water to the total volume of 25 ul. The reaction condition was the same for all genes analyzed: an initial denaturation at 95°C for 2 min, and 40 cycles of 95°C for 15 sec, 58°C for 20 sec, 72°C for 25 sec. Amplification curves were constructed, and the relative expression of mRNA was calculated by 2-ΔΔCq method as previously described. Data was analyzed using GraphPad Prism 7.0.

Results : Analyzing the results obtained showed that temporal lobe epilepsy has no effect on the GS gene expression compared to control animals, while Ceftriaxone treatment can upregulate GS expression compared to that of animals in Pilocarpine group ($p<0.0001$). Furthermore, 31 days post SE, no significant change in GS expression is observed among the groups studied.

Conclusion : TLE can increase excitotoxicity and consequently cause severe damage to hippocampus. In this study, it is demonstrated that decreasing glutamate excitotoxicity by targeting astrocytic glutamate clearance can upregulate GS expression in short term and thus improve the glutamate-glutamine cycle. This improvement can further increase the glutamine needed for GABA synthesis. It is believed that malfunctioning of this cycle can severely damage synaptic activity and thus cause hyperexcitability.

Keywords : Epilepsy, GS, GLT-1, astrocyte