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Effect of astrocytes protease-activated receptors (PARs) stimulation on cultured astrocytes derived from rat brain

*S. Hosseini¹,², M. Fereidoni¹, F. Sabouni²

¹Ferdowsi University of Mashhad, Biology, Mashhad, Islamic Republic of Iran
²National Research Institute of Genetic Engineering and Biotechnology, Tehran, Islamic Republic of Iran

Introduction: Astrocytes are more abundant cells within the central nervous system even to 50% which present in a spectrum of disorders including brain injuries, ischemia, infections, autoimmune and neurodegenerative diseases. At pathologic states they produce inflammatory cytokines, chemokines, ROS, NO and sometimes apoptosis. They have protease-activated receptors (PARs) can activated by proteases such as thrombin and trypsin which are important in brain inflammation. The study is aimed to investigate the effects of trypsin at different concentrations (1 to 100 U/ml) on the cultured astrocytes.

Methods: Brains of 2 days rat infants were derived and then homogenized after meninges removal, homogenizes were cultured in DMEM+10%FBS medium. 10 days later astrocytes were harvested and re cultivated for more purifications to 95% (using Immunocytochemistry method) to employ for tests. They were affected by different concentrations of trypsin (1, 5, 10, 15, 20, 40, 60, 80 and 100 U/ml) and to reveal the inflammation progress, NO concentrations (test of Griess) were assessed after 24 and 48 hours.

Results: Trypsin to 20 U/ml had significant inflammatory and NO rising effect with a dose dependent manner on cultured astrocytes (p<0.001) as trypsin 20 U/ml increased NO elevation two fold more than control (p<0.001). At higher concentration than 20 U/ml, NO elevation effect was diminished (p<0.001). as even at 100 U/ml, NO production was fewer than control (p<0.001).

Conclusion: Inflammatory effects of trypsin at 5 to 20 U/ml may be due to the stimulation of astrocytes PAR-2 receptors and then increasing the activation of NF-κB, PKC, MAPKs like as ERK, P38 and JNK, so it increases the iNOS activation then NO production. But higher trypsin concentration possibly made astrocytes apoptosis then NO production diminished. This assumption is needed to investigate more.