

Understanding the effects of MG132, MLN4924, Bicuculline, and AP-5 on Neuron Cell Cultures

Sarisha Mahajan¹, Bin Luo², and Lin Mei, MD, PhD²

¹Revere High School, Richfield, OH

²Department of Neuroscience, Case Western Reserve University, School of Medicine, Cleveland OH

Background:

Neuronal cultures are used in labs to study the normal physiology of the nervous system, and to study the effects of diseases in the nervous systems and drugs used to treat those conditions. Neuronal cultures allow us to study the effects of different chemicals on the growth of the cell. The four chemicals used in this experiment are as follows: MG132 is a ubiquitin-proteasome inhibitor, belonging to the class of synthetic peptide aldehydes. It blocks the breakdown of proteins and is used to prevent cells from transitioning into anaphase. MLN4924, or Pevonedistat, is a small molecule, inhibitor of NEDD8-Activating Enzyme (NAE). Bicuculline is a light-sensitive competitive antagonist of GABA_A receptors. AP-5 is a selective NMDA receptor antagonist that inhibits their ligand binding site. It is useful to isolate the action of other glutamate receptors in the brain.

Goal: To examine the effects of various chemicals on the growth of neuron cells cultured *in vitro*.

Methods and Materials: A pregnant mouse was euthanized and the brains of the E18.5 mice were removed. The cortices were separated from the rest of the brain, minced, and broken down into individual cells with trypsinization medium. The cells were then added to neurobasal medium and grown *in vitro* in a 5% CO₂ and 37-degree-Celsius incubator. On DIV 5, four chemicals; 10 uM of MG132 and MLN4924; 30uM of Bicuculline, and 100uM of AP-5; were added to the medium. The cell growth was monitored for 24 hours and the pyramidal neurons were stained to examine the effects.

Results: As MG132 and MLN4924 are the inhibitors of protein degradation, they obstructed the normal physiological functions of the neuron, creating a shorter and less complex neuron. Bicuculline inhibited GABA transmission which usually dampen the cell, making the pyramidal neurons more active and complex. Since AP-5 blocked the glutamate transmission, it was expected that the neuron would become less active, but interestingly, we did not see any differences when it was compared with the control neuron.

Conclusion: We have found that after the treatment of MG132 and MLN4924, apoptosis occurred in the cell, decreasing the total length and intricacy of the neurons. The Bicuculline treatment resulted in longer branches and a more elaborate neuron, but the AP-5 treatment surprisingly, showed no obvious changes in the growth of the neuron.