**Background**

*Clomipramine* is a tricyclic antidepressant (TCA) that is used clinically to treat mood and depressive disorders, as well as anxiety disorders. Research suggests that clomipramine also possesses potent apoptotic activity in different cell lines in vitro.

*Astrocytoma* is a malignant type of brain and spinal cancer; stemming from astrocytes, the major type of glial brain cells. *Western Blot* is used to determine the presence and amount of specific proteins with specific antibodies and using a peroxidase-based staining reaction.

**Goal of the Study:** To test the effect of clomipramine on the proliferation of astrocytoma cells, and to determine whether this antidepressant may also affect the motility astrocytoma cells by modifying their cytoskeletal proteins.

**Experimental Design:** Astrocytoma cells were used to test the effect of clomipramine at various doses and time points and in different culture medium. These experiments were performed in cell culture by monitoring cell numbers at various time points and doses. Western Blot then was conducted to determine to effects of clomipramine on the amount of the cytoskeletal proteins: nestin, synemin, vimentin, GFAP and actin.

**Western Blot Analysis of Cytoskeletal Proteins**

**Method:** Gel electrophoresis is used to first separate cellular proteins, which are then transferred onto nitrocellulose paper through electric plates in a conducting buffer. After the protein transfer, the blotted membrane is blocked with milk to prevent any nonspecific binding of antibodies to the surface of the membrane. Primary and secondary antibodies against the proteins of interest are incubated on the membrane and the peroxidase activity associated with the secondary antibody is detected using a substrate that generates a brown color when incubated with peroxidase. The color intensity is then quantified with a laser scanner and correlates with the abundance of the protein of interest in a given sample. In our case, the samples were represented by U373 astrocytoma cells treated for 3 days with 0 and 5 µM of clomipramine in ITS medium.

**Results:** Quantification of the blots and normalization of the values to actin show that clomipramine treatment increased synemin levels by 70% but reduced nestin and GFAP levels by 40% and 70%, respectively, while vimentin protein levels were not affected.

**Conclusion:** Clomipramine alters cytoskeletal proteins in astrocytoma cells, Since these proteins are key for cell motility, this raises the possibility that clomipramine may reduce the spread of astrocytoma cells.

**References**


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**Proliferation Assay**

**Method:** U373 astrocytoma cells were plated in 6 well plates and treated with clomipramine. The number of cells was counted with a hemocytometer after trypsinization to determine the effect of 0.5, 1, 5 and 10 µM clomipramine at 1, 2, and 4 days after adding the compound. T-test statistical analysis was performed and asterisks indicate results significant at p<0.005 or better.

**Results:**

**Conclusion and Future Work**

Out results show for the first time that clomipramine not only suppresses the proliferation of astrocytoma cells, but also that it alters the levels of various cytoskeletal proteins important for metastasis. This suggests, that clomipramine in addition to its current use to treat depression may also show promise for the treatment of certain cancers. Future studies will be conducted to consolidate these results through rigorous statistical analysis.

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