Establishing the cerebellar granule neuron and astrocyte primary culture model for MeHg toxicity studies

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Background

Methylmercury (MeHg), found in commonly contaminated food sources such as fish, is a neurotoxin known to cause many physiological effects. Although many acute toxic effects of MeHg are known, the molecular mechanisms of its toxicity on neurons remain unclear.

The toxicity of MeHg is characterized by loss of neurons in the cerebellum resulting in symptoms such as ataxia, weakness in the extremities and sensory disturbances. In fact, cerebellar granule neurons, have been shown to be particularly sensitive to MeHg.

Traditionally, astrocytes were believed to only provide a supportive function to neurons. However, recent literature suggest new physiological roles for these cells. For example, growth factor second messengers (e.g. IP3, cAMP) are communicated through gap junctions present between astrocytes and neurons.

The primary culture model is often used for the study of neurotoxic effects of chemical compounds. Co-cultures, as well as mono-cultures, may provide useful tools for the study of interactions between cerebellum granule neurons and astrocytes in MeHg toxicity.

Objective

Establish primary cerebellum granule neuron and astrocyte mono and co-culture models for MeHg toxicity studies.

Methodology

1) Cerebellar granule neuron culture

- Cerebellum dissected from post-natal 4-6 days old Balb-c mice
- Single cell suspension obtained using the Papain Dissociation System (Worthington)
- As suggested by Lee 2009, incubation of cell suspension on PDL coated plates permits the removal of glial cells.
- Culture grown in vitro for 7-10 days

2) Cerebellar astrocyte culture

- Cerebellar tissue dissected from post-natal 4-6 days old Balb-c mice and dissociated using trypsinization method
- Astrocytes isolated using Anti-GLAST (ACSA-1) Micro bead Kit (Miltenyi Biotec)
- Culture grown in vitro for 10-14 days.

3) Co-culture model

- Cerebellar granule neurons cultured on astrocyte monolayer at a cell ratio of 1:4 to 1:5 (astrocytes: neurons)
- Co-culture grown in vitro for 7 days.

4) MeHg exposure

- MeHg from stock solution diluted into medium at varying concentrations (0-20uM)
- All primary mono and co-cultures exposed to MeHg for 24h and 48h

Results

1) Immunofluorescence

Mono-culture models

Astrocytes and neurons plated on PDL coated coverslips and maintained in vitro for 7-10 days.

Cerebellar granule neuron monoculture stained using MAP2 with Cy3 and cerebellar astrocytes monoculture GFAP with FITC. All cells stained with DAPI.

Images captured using fluorescence microscope with 400x magnification.

Co-culture model

Astrocytes seeded on PDL coated coverslips followed by cerebellar granule neurons. The co-culture was maintained in vitro for 7 days.

Cerebellar neurons stained using MAP2 with Cy3 and cerebellar astrocytes with GFAP with FITC. All cells stained with DAPI.

Images captured using fluorescence microscope with 400X magnification.

Discussion

The proposed primary culture model provides a good model for MeHg neurotoxicity studies. Immunofluorescence staining demonstrates cell purity by the overlap of DAPI stained nuclei with either the astrocyte GFAP or neuron MAP2 marker. However, flow cytometry will be further used to obtain quantified purification data.

MTT results show that MeHg induced both a time and dose dependent decrease in cell viability. Significant cell death observed at concentrations over 1.25uM in all cultures and time points. Of note, the exception to this was 24h neuron mono-culture and co-culture at respectively 5uM MeHg. These results provide a range of concentrations to be used in future studies.

In summary, the present primary culture methodology permits the study of MeHg toxicity in monoculture and the interactions that may occur in the co-culture model. In addition, it may also provide a model for other neurotoxin/chemical studies.

Conclusion

In this study we successfully established the primary culture model. The proposed methodology and cell viability test facilitates our future MeHg studies.

References


Acknowledgements

This research was made possible thanks to grants from UROP, University of Ottawa, and the USRA, NSERC. Special thanks to Dr. Laurie Chan and Yueting Shao for all the support and direction given to this project. I also thank the technical support received from the CAREG laboratories and Vanier Hall animal facility technicians.