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.[1] In fact, cerebellum 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 (eg. IP3, cAMP) are communicated through gap junctions present between astrocytes and neurons.[2]

The primary culture model is often used for the study of neurotoxic effects of chemical compounds.[3] 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 day old Balb-c mice
- Single cell suspension obtained using the Papain Dissociation System (Worthington)
- As suggested by Lee 2009[4], incubation of cell suspension on PDL coated plates permits the removal of glial cells.
- Culture grown in-vitro for 7-10 days

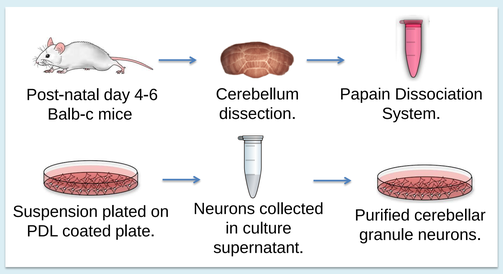


Figure 1 : Cerebellar granule neuron isolation

2) Cerebellar astrocyte culture

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

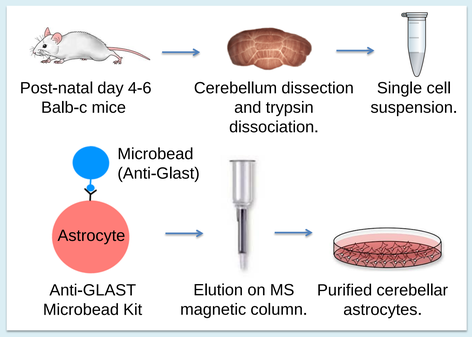


Figure 2 : Cerebellar astrocyte isolation

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.

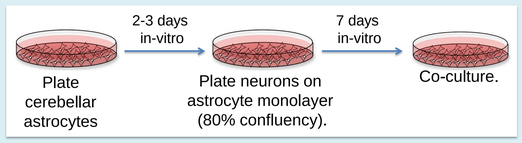


Figure 3 : Co-culture plating

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

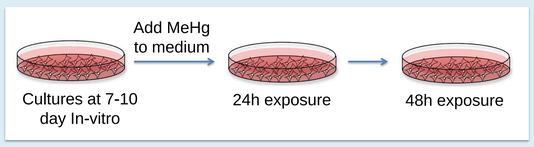


Figure 4 : Methyl mercury exposure

2) Cell viability - MTT assay

Cell viability measured using an MTT assay. Cells were collected on post-natal day 4 and plated on 96 well plates. Mono and co-cultures dosed on in-vitro day 7.

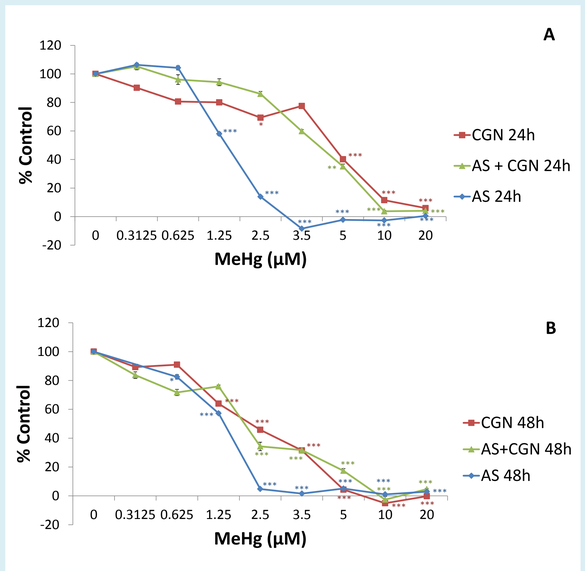


Figure 5 : Cell viability of cerebellar granule neurons and astrocytes in mono and co-cultures following a A) 24h exposure and B) 48h exposure to varying concentrations of MeHg. (n=5-6, One-way ANOVA Tukey's test, *p<0.05, **p<0.01, *** p<0.001)

Results

1) Immunofluorescence

Mono-culture models

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

Cerebellum granule neuron mono-culture stained using MAP2 with Cy3 and cerebellar astrocytes mono-culture GFAP with FITC. All cells stained with DAPI.

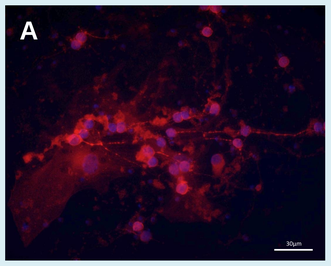
Images captured using fluorescence microscope with 400x magnification.

Co-culture model

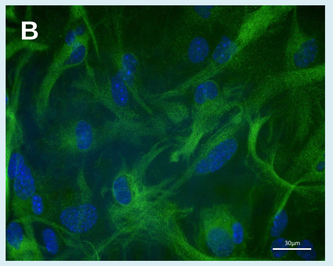
Astrocytes seeded on PDL coated coverslips followed by cerebellar granule neurons. The co-culture was maintained in-vitro for 7 days.

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

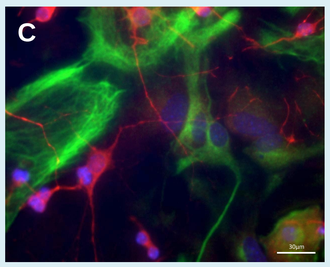
Images captured using fluorescence microscope with 400X magnification.

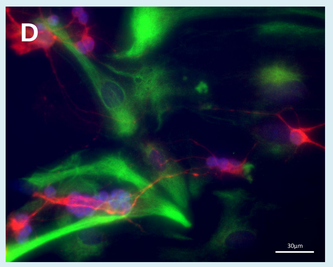


A Neuron monoculture : Cerebellum granule neurons (red) with nuclei (blue).



B Astrocyte monoculture : Cerebellar astrocytes (green) with nuclei (blue).





C-D Astrocyte and neuron co-culture: Astrocytes (green) and cerebellar granule neurons (red) with nuclei (blue).

Discussion

The proposed primary culture provides a good model for MeHg neurotoxicity studies. Immunofluoresence 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.

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