



# Aggregating Brain Cell Cultures: Investigation of Stroke Related Brain Damage

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## Summary

*Aggregating brain cell cultures are three-dimensional primary cell cultures derived from embryonal rat brain cells. Within 3-4 weeks in culture under continuous agitation, they exhibit organotypic structures and functions. The transient arrest of*

*agitation induced adverse effects comparable with those occurring in the ischemic brain in vivo. This culture system therefore offers a suitable in vitro model for the elucidation of ischemia-related pathogenic processes in the brain.*

*Keywords: brain, aggregate culture, ischemia*

## Background Information

### Stroke related brain damage

*The risk of a cerebrovascular accident such as a stroke doubles every 10 years from the age of 45 onwards. At the age of 75, the incidence reaches about 2%. Ischemia-related brain damage is thus one of the most important health problems in industrialised countries. This situation is likely to become further aggravated as the average age of the population continues to rise.*

*The extent of brain tissue damage depends on the brain region affected as well as on the duration and degree of decreased tissue blood supply. In regions of incomplete ischemia, selective neuronal damage and delayed cell death are observed. A promising therapeutic strategy would be to develop medications which can interrupt early stages in the neuro-degenerative cascade. To find such medications, the cellular and molecular reactions involved in pathogenicity need to be fully elucidated. Accordingly, research in this area has been given high priority, in both areas of basic research and drug development.*

## The brain is highly vulnerable to ischemia

The interruption of blood supply to the brain (ischemia) deprives brain cells of glucose and oxygen, causing irreversible brain damage within minutes. The brain is particularly vulnerable to ischemia because of 1) the very high rate of oxidative metabolism in this organ, requiring a continuous supply of oxygen and glucose, 2) the metabolic interdependence of the two types of brain cells, neurons and astrocytes, and 3) the sensitivity of neurons to changes in ion homeostasis brought on by ischemia.

Cells in ischemic brain tissue undergo a number of changes: they rapidly lose their energy stores, their membranes become depolarised, calcium loads increase, reactive oxygen species are produced and excitotoxic effects are found. These biochemical changes are followed by irreversible structural changes and cell death by apoptosis and/or necrosis. The neurons are particularly sensitive to injury, whereas the reactivity of glial cells (astrocytes and microglia) tend to amplify and propagate the adverse effects.

## Animal models versus cell cultures

Today, research on ischemia relies mainly on animal models. Unfortunately, such studies involve a high degree of discomfort for the animal. *In vivo* approaches involve procedures such as the surgical occlusion of major arteries, or (less invasive) the induction of reproducible infarcts in a selected area of the brain by means of artificially induced thrombosis. The animal models appear to closely reproduce the characteristics of gray matter ischemic injury in humans; however, the patterns of pathology in the body resulting from ischemia in a particular region of the brain can be quite different than those seen in humans.

Diverse cell culture models have been developed to study mechanistic aspects of cerebral ischemia. These include monolayer cell cultures and three-dimensional cultures such as aggregated brain cells and brain tissue slices. Each model has specific advantages and disadvantages (Tab. 1). However, neither animal models nor culture systems are able to reproduce all the characteristics of ischemic pathologies encountered in humans.

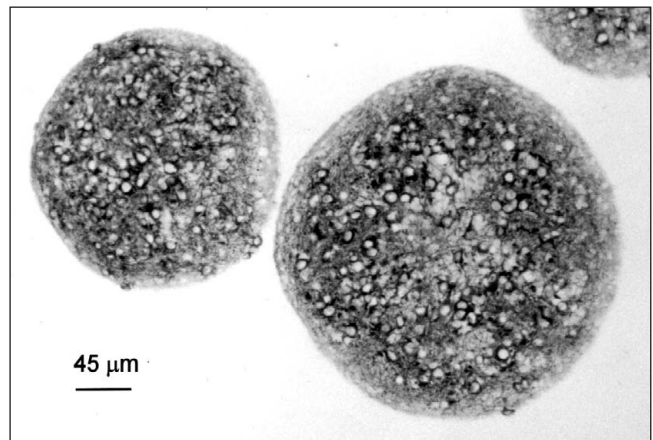
### Ischemic pathways can be induced....

Aggregating brain cell cultures are prepared from embryonic rat or mouse brain tissue (Honegger and Pardo, 1999). The cells can be maintained for several weeks as floating (suspension) cultures under continuous gyratory agitation (swirling at 80 rpm) in an incubator (Fig. 1).

Ischemic conditions can be induced in the cultured aggregates simply by stopping the gyratory agitation for different lengths of time, and then restoring again normal culture conditions (Pardo and Honegger, 1999a). The effects of transiently stopping the circulation of medium and air to the cells are assayed one week after the insult. Using cell-type specific biochemical markers of normal brain cell function (for example, enzyme activities such as glutamic acid decarboxylase (GAD) and glutamine synthetase), it was found that the transient immobilisation caused selective neuronal cell death and delayed glial reactions similar to those found in incomplete ischemia *in vivo* (Pardo and Honegger, 1999b). Similar adverse effects were observed in cultures subjected to hypoxia or hypoglycemia (Pardo and Honegger, 2000). The latter condition also showed significant alterations in the metabolism of amino acids and the accumulation of neurotoxic ammonia (Honegger et al., 2002).

### ....and prevented

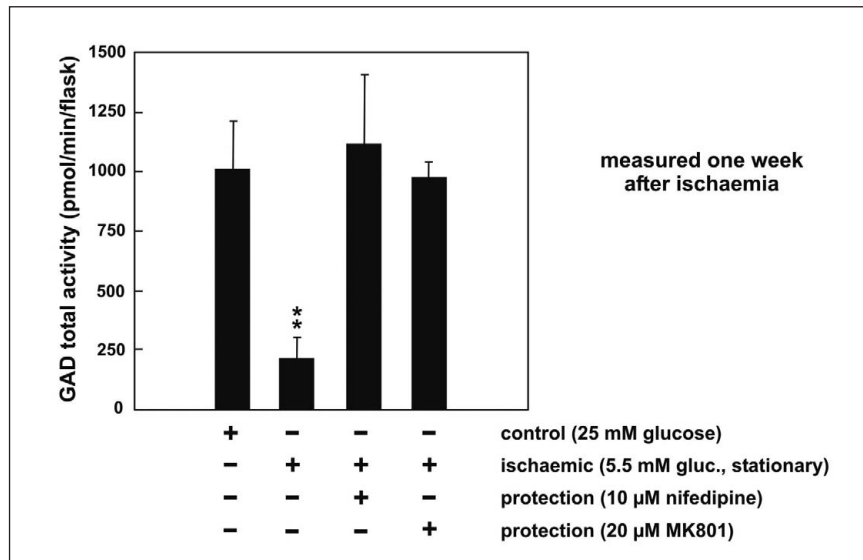
Glutamate stimulation and excessive calcium influx are thought to be critical events in ischemia-induced neurotoxicity. Accordingly, it might be possible to protect neurons by inhibiting these early steps along the ischemic cascade. Our results



**Fig. 1: Each flask contains over a thousand of spherical cell aggregates.** Sections of differentiated aggregates showing stained neurons – cell bodies and neurites stained for microtubule associated protein (MAP-2), surrounded by unstained glial cells.

**Tab. 1 Comparison of *in vitro* culture models**

<i>In Vitro</i> Model	Specific advantages	Disadvantages
<b>Monolayers</b>		
tumor cells	<ul style="list-style-type: none"> <li>- defined cell population</li> <li>- direct access for manipulations</li> </ul>	<ul style="list-style-type: none"> <li>- high variability</li> <li>- transformed cells</li> </ul>
embryonic brain cells	<ul style="list-style-type: none"> <li>- direct access for study of morphology immunocytochemistry</li> <li>- biochemical analyses</li> </ul>	<ul style="list-style-type: none"> <li>- partial loss of brain specific functions</li> <li>- ischaemia inducible only under harsh conditions</li> <li>- limited cell-cell interactions</li> </ul>
<b>Cell Aggregates</b>		
3D-cultures with brain embryonic cells and cell-cell interactions	<ul style="list-style-type: none"> <li>- high yield and reproducibility</li> <li>- undergo extensive maturation</li> <li>- cell-cell interactions involved in cascade of ischemia induced adverse effects</li> </ul>	<ul style="list-style-type: none"> <li>- limited access for microscopic observations and electrophysiology</li> </ul>
<b>Tissue Slices</b>		
from postnatal brain	<ul style="list-style-type: none"> <li>- preserve to some extent the organotypic structure of brain regions</li> <li>- direct access for manipulations</li> </ul>	<ul style="list-style-type: none"> <li>- low yield and low reproducibility</li> <li>- repetitive sampling not possible</li> </ul>



**Fig. 2: Protection from ischemia induced neurotoxicity by a calcium channel blocker (nifedipine) or an NMDA receptor antagonist (MK801). GAD activity is used as a marker of normal brain cell function.**

with aggregating brain cell cultures indicate that this is indeed the case: Nifedipine, a blocker of voltage-gated calcium channels, and MK801, an antagonist of the NMDA ionotropic glutamate receptor, were both able to inhibit the loss of GAD activity (Fig. 2).

### Aggregating brain cell cultures, a promising *in vitro* model

In summary, aggregating brain cells exhibit unique features useful for the study of pathogenic mechanisms involved in cerebral ischemia. The culture system permits easy handling, repetitive sampling and post-injury follow-up studies. Data obtained so far demonstrate that many of the fundamental pathogenic processes involved in ischemia can be studied and recognised in aggregating cell cultures. A thorough knowledge of the mechanisms involved in ischemic cell damage will allow us to precisely identify the critical steps and cellular and subcellular targets in this process. This in turn will enable the development and testing of potential therapeutic agents designed to inhibit or counteract specific steps in the ischemic cascade of neurodegeneration.

### References

Honegger, P. and Pardo, B. (1999). Separate neuronal and glial  $\text{Na}^+$ , $\text{K}^+$ -ATPase isoforms regulate glucose utilization in response to membrane depolarization and elevated extracellular potassium. *J. Cereb. Blood Flow Metab.* 19, 1051-1059.

Honegger, P., Braissant, O., Henry, H. et al. (2002). Alteration of amino acid metabolism in neuronal aggregate cultures exposed to hypoglycaemic conditions. *J. Neurochem.* 81, 1141-1151.

Pardo, B. and Honegger, P. (1999a). Aggregating brain cell cultures as a model to study ischemia-induced neurodegeneration. *Toxicology in Vitro* 13, 543-547.

Pardo, B. and Honegger, P. (1999b). Selective neurodegeneration induced in rotation-mediated aggregate cell cultures by a transient switch to stationary culture conditions: a potential model to study ischemia-related pathogenic mechanisms. *Brain Res.* 818, 84-95.

Pardo, B. and Honegger, P. (2000). Evaluation of aggregating brain cell cultures as a model for studying ischemia-related neurodegenerative processes. In: M. Balls, A.-M. van Zeller and M. E. Halder (eds.), *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (241-247). Elsevier Sci.

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