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Apoptotic and Necrotic Processes in a Rat Organotypic Hippocampal Slice Culture Model of Ischemic Stroke without Reperfusion

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APOPTOTIC AND NECROTIC PROCESSES IN A RAT ORGANOTYPIC HIPPOCAMPAL SLICE CULTURE MODEL OF ISCHEMIC STROKE WITHOUT REPERFUSION

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Background

- The use of organotypic hippocampal slice cultures (OHSC) has become a powerful tool for studying cell damage.[5]
- This model reproduces basic morphological and functional properties of the hippocampus and preserves the intimate neuron-glia interactions.[2,5]
- The hippocampus is one of the most extensively studied areas of the brain as it is the most susceptible brain region for damage from ischemia and hypoxia.[1]
- The flexibility of organotypic hippocampal slices has allowed the study of various experiential conditions such as hypoxia and ischemia and provided treatment strategies for neurodegenerative disorders like stroke.[2,3]
- Detection of neuronal cell death is a standard procedure to assess the severity of damage in tissue culture models of neurodegenerative diseases. However, information about the time course of injury during an ischemic event is limited and experiments documenting apoptotic processes that occur in the ischemic penumbra, where cells are still salvageable, are lacking

Objectives

- Examine the time course of apoptosis and necrosis in a rat OHSC model of ischemia using oxygen-glucose deprivation (OGD) without reperfusion.

Materials and Methods

Organotypic Hippocampal Slice Collection:

- This study is in compliance with NIH animal care guidelines and was approved by the Purdue Animal Care and Use Committee.
- Hippocampal organotypic slice cultures were prepared as described by Stoppini et al. (1991) with minor changes.[4]
- 6-9 day old rat pups were anesthetized and sacrificed.
- Hippocampi were removed and sliced in 400 µm increments.
- Slices were cultured on 0.4 µm Millpore inserts in organotypic media and incubated at 37˚C and 5% CO_2/95% air in a humidified atmosphere.
- The media was changed twice weekly.

Oxygen-Glucose Deprivation Timeline

- 7-10 days in vitro (DIV) slices were exposed to hypoxia (1% O_2 and 5% CO_2 in nitrogen) and glucose deprivation at 37°C for periods ranging from 0, 2, 4, 8 and 16 hours to mimic conditions following cerebral ischemia.
- Cell damage was assessed by staining immediately following OGD to avoid reperfusion effects.
- The viability of the hippocampal slices exposed to OGD was determined qualitatively and quantitatively using YO-PRO 1 iodide, which stains apoptotic cells, and propidium iodide (PI), which stains necrotic cells.
- All analytical tests were run immediately after OGD to avoid reperfusion effects.
- Controls consisted of slices maintained in organotypic media under normoxia.

Results

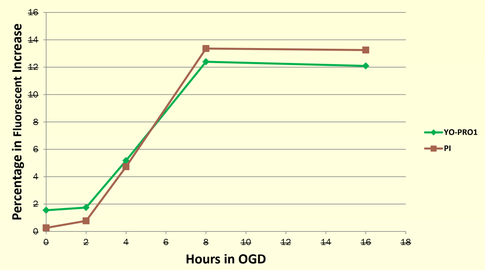

Figure 1: Average percent in YO-PRO1 and PI staining through multiple experiments for time periods ranging from 0, 2, 4, 8 and 16 hours.

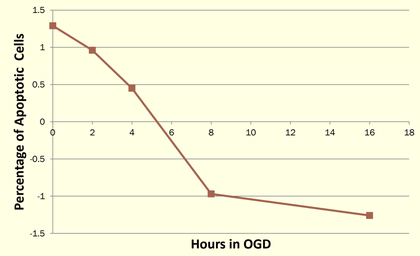

Figure 2: Average percent in apoptotic staining through multiple experiments for time periods ranging from 0, 2, 4, 8 and 16 hours.

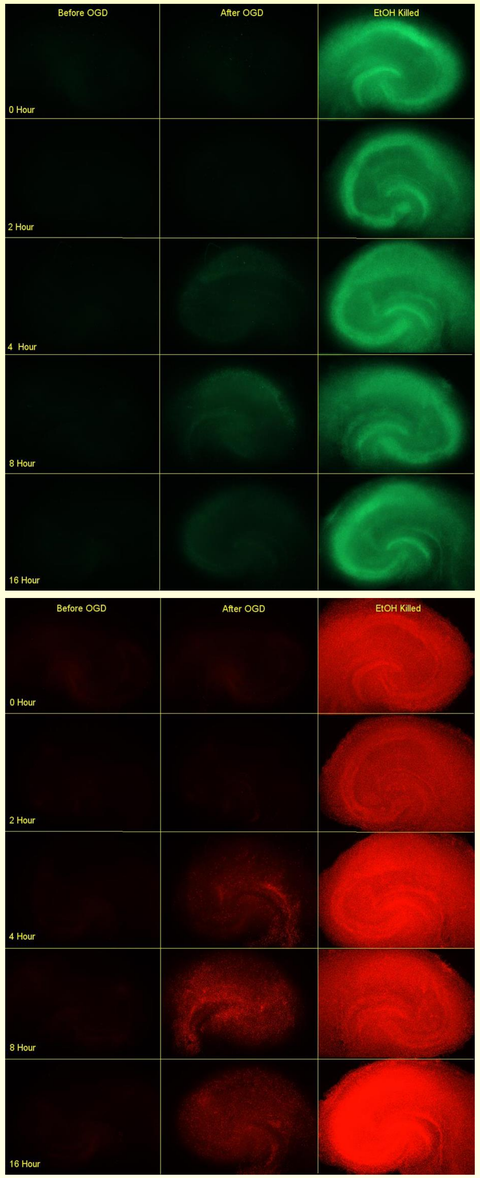

Figure 3: Shows YO-PRO1 and PI staining throughout 0, 2, 4, 8 and 16 hours. Taken a 5x magnification,

- Apoptosis in the OHCs increased gradually with increased time of OGD.
- Image analysis of YO-PRO 1 iodide fluorescent intensity showed that after 1 hour of OGD an increase in YO-PRO 1 staining was already apparent in the CA1 and dentate gyrus with maximum staining intensity occurring between 4 and 8 hours of OGD.
- In parallel, quantitative analysis showed a small number of PI stained cells which could also be detected as early as 1 hour following OGD, but notable increases were not observed until at least 6 hours of OGD.
- Along with the changes in fluorescent intensity of the two viability stains, there were observed gross changes in the morphology of the slice under light microscopy.

Conclusions

- The data gathered suggest that the most suitable length of ischemia without reperfusion for the maximization of apoptosis in our OHC model is between 4 and 8 hours.
- Within the 4-8 hours time frame, large increases in apoptotic cells which are potentially salvageable are can be easily accessible for application and assessment of neuroprotective interventions.
- It provides a unique opportunity (1) to select the severity of the ischemic insult by varying the period of OGD, (2) to estimate the amount of damage by evaluating the effects on viability through generation of apoptotic and necrotic cells, and (3) to test potential new treatments for hypoxic-ischemic stroke.

References

1. Holopainen I. E. (2005). Neurochemical Research, *30*(12) 1521-1528.
2. Noraberg et al (2005). Current Drug Targets, (4), 435-452.
3. Peña F. (2010). Current Medicinal Chemistry, 17. 987-1001.
4. Stoppini et al. (1991). *J. of Neuroscience Methods*, *37*, 173-182.
5. Su et al (2011). Brain Research 1385, 68-76 .

Acknowledgements

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