NEUROPROTECTION STRATEGIES TOWARDS NEONATAL STROKE

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INTRODUCTION

Neonatal hypoxia-ischemia (HI) is a type of stroke that affects the brain at early stages of development, from birth until postnatal day 28. It is a major cause of death and disability in the paediatric population worldwide; long-term sequelae include behavioural and learning disabilities, cerebral palsy and motor dysfunction.

In neonatal HI, **oxygen deprivation** affects neurons, but also astroglia, microglia and myelinisation. Since brain injury is highly associated to oxidative stress and there is currently no effective treatment, **antioxidant therapy** has been proposed as a novel approach to prevent and reduce brain damage.

Previous preclinical *in vivo* studies have shown that nutraceuticals, including **polyphenols**, have neuroprotective properties against HI-induced brain damage. Polyphenols present known antiapoptotic, anti-inflammatory and antioxidant properties, and are interesting candidates to be tested against HI pathophysiology.

Our lab is interested in evaluating the **neuroprotective activity** of a plant-derived phenolic compound.

AIMS

- Validating the HI mouse model comparing brain damage in control and HI groups.
- Verifying our model is suitable to represent the reduction in myelinisation and the increase in astroglia and microglia activation observed in neonatal stroke.
- Evaluating the neuroprotective potential of the phenolic compound based on the reduction in brain damage in treated versus non-treated groups.
- Optimising a screening model in *Drosophila melanogaster* larvae for the evaluation of different potential neuroprotective compounds against neonatal stroke.

METHODS

MOUSE MODEL

Our group uses the Rice-Vannucci hypoxic-ischemic mouse model by **ligation of the left common carotid artery and hypoxia** in 7 day-old pups. We inject the phenolic compound intraperitoneally 20 min before the ligation. After 2 h of recovery, pups are exposed to 90 min hypoxia (9% O_2). The brain is dissected a week later to test the neuroprotective effects by histology and immunohistochemistry to study astroglia and microglia activation and myelinisation (using GFAP, Iba1, and MBP respectively).

We have developed a **histological scoring system** based on histology images to categorize brain damage in different cerebral structures (0-28 points, from no brain damage to intense damage, respectively).

In parallel, we obtained **brain and plasma samples** to analyse the concentration of the phenolic compound at different time points by mass spectrometry.

DROSOPHILA SCREENING MODEL

A screening *in vivo* model using *Drosophila melanogaster* has also been developed to optimise the analysis of the neuroprotective properties of this and other phenolic compounds. *Drosophila* **larvae diet** is supplemented with the compound twice. After hatching, 2 day-old flies are exposed to 2.5 h severe hypoxia (1% O_2). **Mortality** is assessed daily during the 3 days following hypoxia whereas **locomotor activity** is analysed with a Drosophila Activity Monitoring system during 4 h of recovery.

RESULTS

NEONATAL HYPOXIA-ISCHEMIA MOUSE MODEL

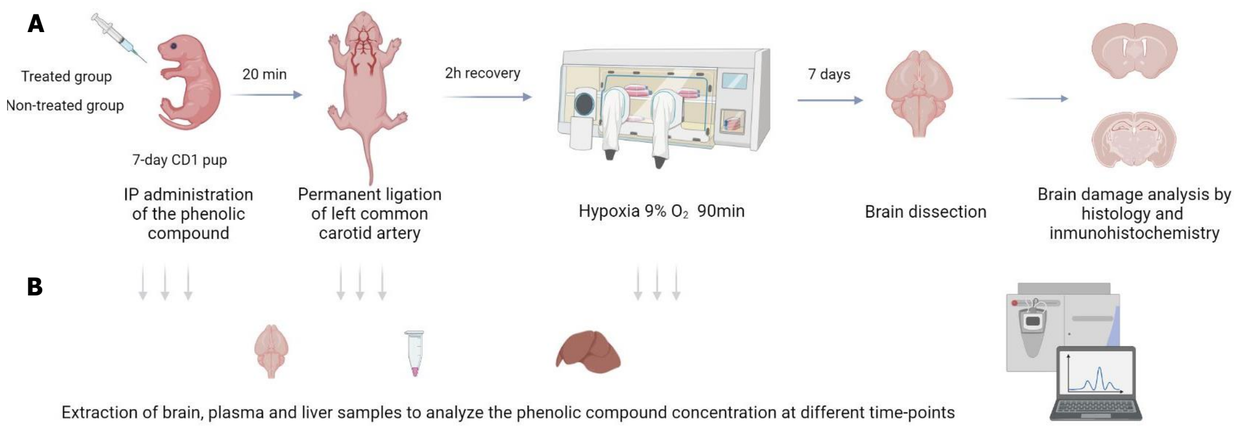

Figure 1. (A) Neuroprotection study. Seven days after permanent ligation of the left common carotid artery and hypoxia, the brain is dissected and analysed by histology and immunohistochemistry to test the neuroprotective properties of the phenolic compound. (B) Analysis of the concentration of the phenolic compound at different time points by mass spectophotometry in brain, plasma and liver samples.

REDUCED MYELINIZATION IN THE NEONATAL HI MOUSE MODEL

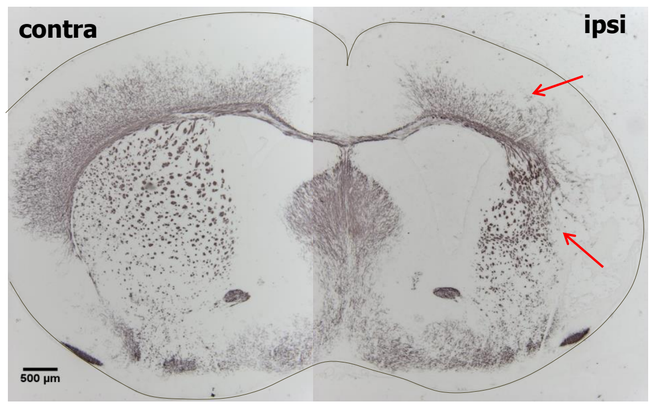

Figure 2. Immunohistochemistry against myelin basic protein (MBP). Myelinisation is reduced in the ipsilateral hemisphere (where the carotid is ligated; right) compared to the control contralateral hemisphere (left).

BRAIN DAMAGE IN THE NEONATAL HI MOUSE MODEL

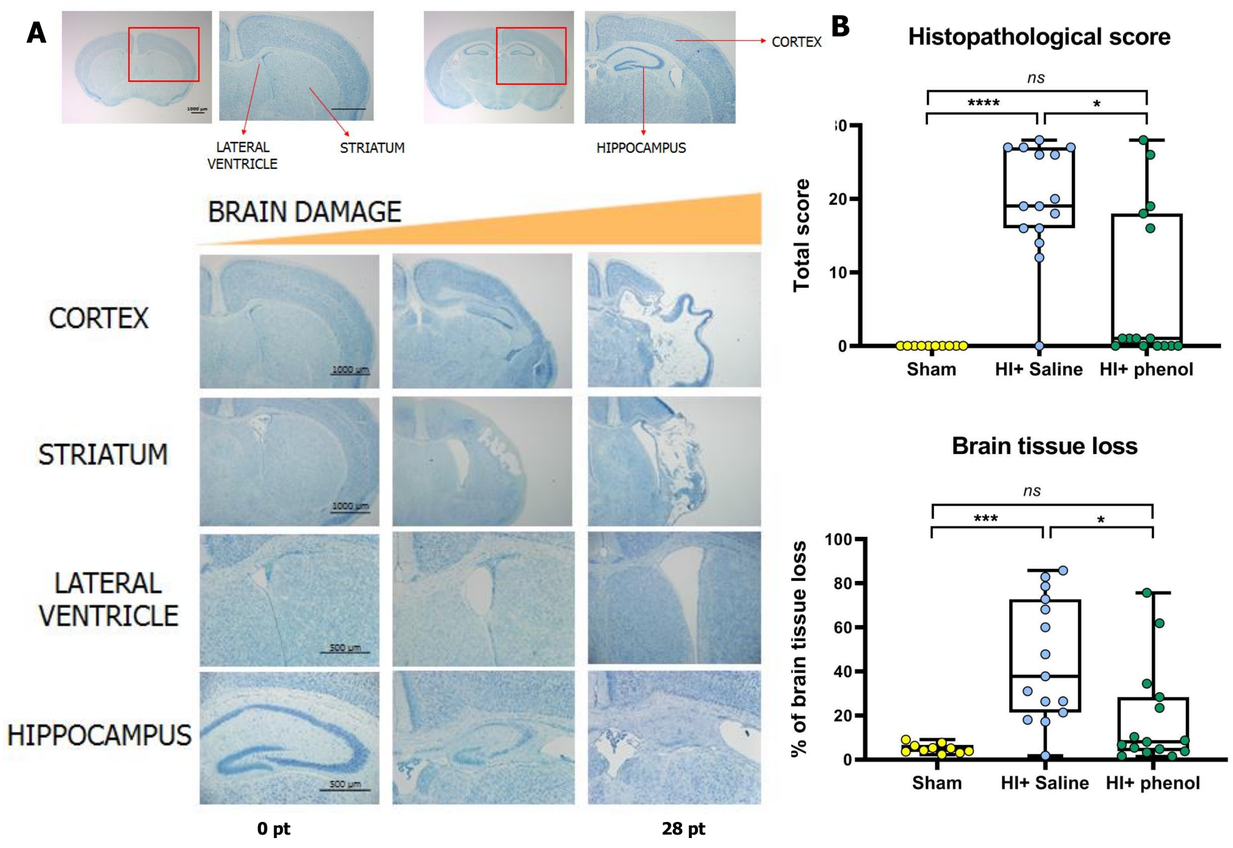

Figure 2. (A) Histopathological score is used to categorise brain damage in the cortex, striatum, lateral ventricle and hippocampus. (B) Non-treated HI group shows increased brain tissue loss and histopathological score than the HI phenol-treated group. 1-way ANOVA Brown-Forsythe, *n* = 10-15 per group: *ns*, non-significant, (*) $p<0.05$, (***) $p<0.001$ and (****) $p<0.0001$.

DROSOPHILA MELANOGASTER LARVAE SCREENING MODEL

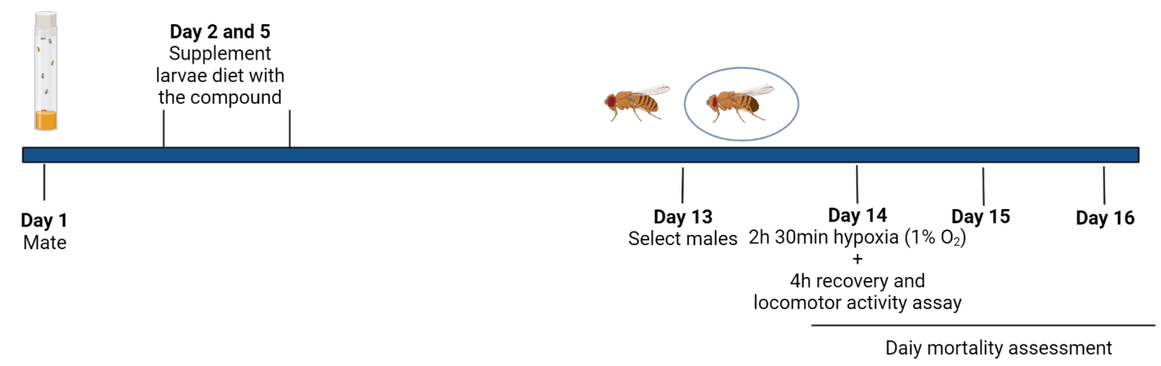

Figure 4. *Drosophila* larvae can be used as a *in vivo* screening model for neuroprotective compounds. Larvae diet is supplemented with the substance and, after hatching, males are exposed to hipoxia. Locomotor activity and mortality are studied.

CONCLUSIONS

- The neonatal HI mouse model is appropriate to study neonatal stroke since it recapitulates brain damage and reduced myelinisation observed in the disease.
- Preliminary results suggest this plant-derived phenolic compound has neuroprotective properties, since it can reduce brain damage after a hipoxia-ischemia event.
- A *Drosophila* larvae screening model that shows increased mortaility and reduced locomotor activity after hypoxia has been set up and can be used to test this phenolic compound and other potential neuroprotective compounds.

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