Combining hypothermia and oleuropein protects white matter in a swine model of neonatal brain hypoxic-ischemic encephalopathy

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Introduction

- Neonatal hypoxic-ischemic encephalopathy (HIE) from birth asphyxia causes nearly one million neonatal deaths worldwide each year.
- The standard of clinical care for HIE is therapeutic hypothermia. However, hypothermia is not fully protective.
- Oleuropein (OLE) is a botanical compound that increases cellular damaged protein clearance and modulates oxidative stress and inflammation.
- We theorized that OLE combined with hypothermia would improve white matter protection above that from hypothermia alone after HI. We also tested whether OLE offers additional benefit in putamen and cortex.

Methods

- Piglets were randomized to 1 of 4 treatment groups: hypoxia-ischemia (HI) + OLE; HI + vehicle; sham procedure + OLE; or sham procedure + vehicle. All groups received overnight hypothermia and rewarming.
- Injury in the subcortical white matter of motor gyrus, corpus callosum, internal capsule, putamen, and motor cortex were measured by western blot, Luxol fast blue stains of myelin density, and neuropathology.
- Friedman RM ANOVA on ranks with post-hoc Holm-Sidak tests.

Results

- Figure 1: Western blots in subcortical white matter. A) Oligodendrocyte marker Olig2 levels differed (p<0.001). Post-hoc tests showed that the HI OLE piglets had greater Olig2 than all other groups (*p<0.05). B, C) Myelinating oligodendrocyte glycoprotein (MOG) and glial fibrillary acidic protein (GFAP) did not differ. D) Example blots.

- Figure 2: Luxol fast blue measures of myelin density A) in the subcortical white matter of the motor gyrus (outlined). B) HI and OLE interactively affected myelin density (p=0.022). In post-hoc tests, OLE increased myelin density after HI compared to vehicle (*p=0.025).

- Figure 3: A) The arrowheads show bridging integrator-1 (BIN1)+ myelinating oligodendrocytes. Arrows show BIN1-negative glia. B) In subcortical white matter, HI and OLE interactively affected the number BIN1+ myelinating oligodendrocytes (p=0.001), and HI (p=0.006) had an independent effect. In post-hoc tests, HI+vehicle reduced the number of myelinating oligodendrocytes to below that of sham vehicle. HI+OLE increased the number of myelinating oligodendrocytes to exceed HI+vehicle. After sham procedure, OLE reduced the BIN1+ cell count relative to vehicle. *p<0.05.

Conclusions

- We identified OLE as a potential adjuvant treatment to hypothermia that protects myelinating oligodendrocytes and myelin after HI.
- This protection was greatest in the subcortical white matter. OLE did not offer any further protection above that from hypothermia alone in the putamen or cortex.
- OLE and hypothermia in uninjured brain could affect the myelinating oligodendrocyte population, and further studies are needed.

- Oligodendrocyte and myelin markers did not differ in corpus callosum or internal capsule.
- The number of normal neurons did not differ in putamen or motor cortex.