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Hypoxia-Ischemia in the Immature Brain and Mechanisms of Reducing Tissue Damage

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Hypoxia-ischemia (HI) in the Immature Brain and Mechanisms of Reducing Tissue Damage

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Introduction

Stroke can occur in individuals of all age groups and presents a clinical challenge in terms of the damage to brain tissue that it causes. One precipitator for stroke is a hypoxic-ischemic event. *Hypoxia* refers to a partial lack of oxygen in the tissues of the body; *ischemia* is a reduction or cessation of blood flow. The extent of tissue damage is influenced by the location of the injury and the age and developmental stage at which the insult occurs. Damage ranges from selective neuronal necrosis, involving only a number of cells, to infarction, the death of all of the cells in a tissue. A model using young rats has been developed to study the course of perinatal hypoxic-ischemic brain damage, as well as pretreatments to reduce that damage. The 7-day-old rat brain is considered to be analogous to the brain of a 32–36-week-old human infant. In our experiments, we induced hypoxia-ischemia in neonatal rats to observe the effects on the brain; in later experiments, we evaluated the protective effect of hypoxic preconditioning.

Clinical Implications

Statistics from 2000 suggest that incidences of hypoxic-ischemic encephalopathy (HIE) occur in up to 1-6/1000 full-term infants and up to 60% of low-birth-weight (premature) infants. Of those newborns affected by HIE, 50-80% survive the newborn period, and of those that survive, 25% will exhibit permanent neurological handicaps, including spastic motor deficits (cerebral palsy), mental retardation, learning disability, and epilepsy. Infants affected by HIE also show retardation in the growth of their brains and bodies relative to their un-afflicted peers. Currently no standard treatment exists for newborn infants affected by HIE.

Materials & Methods

- Litters of Wistar breed rats were randomized at postnatal day 0 (P0) and were reduced to 10 individuals to ensure that all of the pups received adequate nutrition and gained weight accordingly.
- At P7, the pups underwent permanent unilateral carotid artery ligation to induce cerebral ischemia. After an hour of recovery, the pups were subjected to systemic hypoxia in an incubation chamber with a gas composition of 8% oxygen/92% nitrogen (normoxia is 21% oxygen).
- At P14, the animals were sacrificed, and their brains retrieved and frozen. The brains were sliced by a cryostat in 6um coronal sections. We stained brain slices with hematoxylin and eosin, which stains cellular proteins and nuclei.

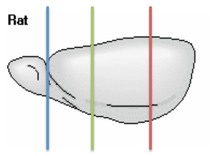
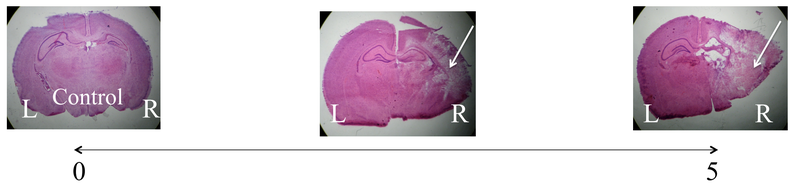


Figure 1. Approximate position of brain slices used in experiments. We removed the olfactory bulbs (blue line) and the cerebellum and brainstem (not pictured). We took sections at both the anterior (green line) and posterior (red line) levels of the brain.

- We examined the brains microscopically and graded ischemic abnormalities according to their severity using a numeric scale, from 0-5. 0 meant no damage, and 5 meant infarction or obliteration of the tissue.



- To precondition with hypoxia, P7 pups were exposed to systemic hypoxia without carotid artery ligation. At P8, the pups underwent unilateral arterial ligation and systemic hypoxia. At P14, the animals were sacrificed and their brains prepared for analysis.

Results

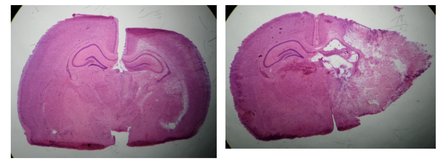


FIGURE 2. A coronal section of the brain at the level of the hippocampus (anterior brain) from P7 rat exposed to HI, stained with hematoxylin-eosin. Both brains show damage on the right side, the hemisphere ipsilateral to the carotid artery ligation. Necrotic or infarcted tissue appears white or less pink and less structured compared to the tissue on the contralateral side.

Results

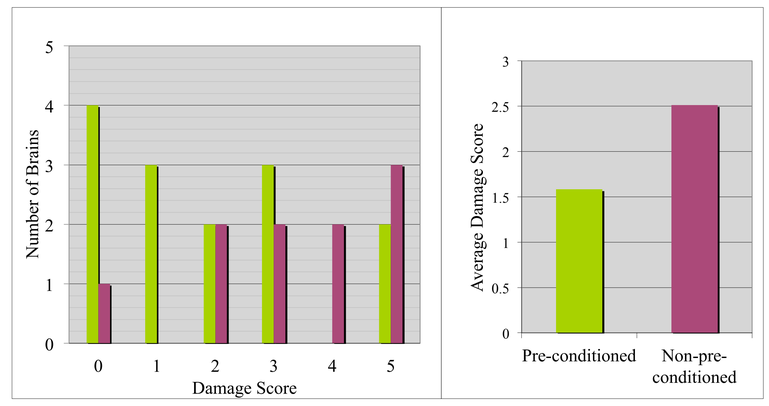


FIGURE 3. Severity of brain damage in rats subjected to unilateral cerebral hypoxia-ischemia with (*green*) versus without (*purple*) hypoxic preconditioning.

Sources

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Conclusions

- Typically, tissue damage occurred with a frequency and severity related to the duration of hypoxia, i.e. HI of longer duration resulted in more brain damage and infarction than did HI of shorter duration. These results were in accord with previously conducted studies.
- Damage scores allowed researchers to compare neurological abnormalities between control and hypoxia-treated groups. Fig. 3 shows that more preconditioned animals had lower damage scores, and more control animals had higher damage scores. These data suggest that preconditioning with hypoxia before a severe hypoxic-ischemic event might reduce the ultimate brain damage.
- Hypoxic preconditioning is thought to provide protection via several different pathways. First, hypoxia stimulates transcription factors to alter gene expression, leading to increased production of red blood cells and blood vessels. These changes lead to increased oxygen carrying capacity, which staves off tissue damage during a subsequent hypoxic event. Another method by which preconditioning might provide protection is the increased production of glycogen (the storage form of glucose). The newly synthesized glycogen acts as an energy reserve during the subsequent hypoxic event, preventing the depletion of high-energy reserves (namely ATP and phosphocreatine) and protecting the animals from secondary energy failure.