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COMMENTARY

Is Erythropoietin the Answer?

Commentary on the article by Fan *et al.* on page 56

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Infants with hypoxic-ischemic (HI) brain injury are at increased risk for death or serious, lifelong neurologic abnormalities. As care of the critically ill newborn has advanced and imaging technologies continue to improve, the incidence and significance of brain injury in the newborn period are becoming clearer. Classically, supportive care was the only option, but the advent of therapeutic hypothermia has created optimism for treatments that can improve long-term function, and efforts to clarify the pattern and timing of injury and potential therapies have intensified.

Increasing evidence suggests that exogenously administered erythropoietin (EPO) has a protective effect in a variety of different models of brain injury. Postinjury treatment protocols in newborn rodents have demonstrated both short- and long-term histological and behavioral improvement. Single- and multiple-dose treatment regimens of EPO after neonatal focal ischemic stroke showed reduced infarct volume (1) and improved short-term sensorimotor (2) and long-term cognitive (3) outcomes. EPO treatment initiated 24 h after neonatal HI also decreased brain injury (4).

EPO potentially benefits brain injured animals by a number of different mechanisms that not only just decrease cell death but also enhance repair. EPO was originally identified for its role in erythropoiesis but has since been found to have other roles. In addition to antiapoptotic effects, EPO plays a vital role in neural differentiation and neurogenesis early in development (5). EPO has also been found to enhance neurogenesis and direct multipotential neural stem cells toward a neuronal cell fate after injury (6). However, a number of cell types besides neurons express EPO and EPO-receptor (EPOR) after injury, including astrocytes, endothelial cells, and microglia (7). Recently, late EPO therapy (48 h after early stroke in the mouse) was found to reorganize white matter and increase oligodendrocyte precursor cells (8). This effect on cell proliferation and cell fate commitment may help explain long-term improvement seen in some injury models and treatment protocols.

In this issue of *Pediatric Research*, Fan *et al.* present evidence that multiple doses of EPO administered over a 48-h period improves behavioral, but not necessarily histological, outcome in a mouse model of neonatal HI. This study included the examination of two different quantities of high-dose EPO (5 kU/kg/dose *versus* 20 kU/kg/dose), each with the same timing of administration after injury (0, 24, and 48 h after completion of HI). Treated animals had increased Ki67+ cells in the subventricular zone and hippocampal dentate gyrus at 72 h after injury, indicating increased progenitor cell proliferation. However, these beneficial effects were only observed in female animals treated with EPO at a dosage of 5 kU/kg. Interestingly, no beneficial effects were observed in male animals or at the increased EPO dose of 20 kU/kg. Similarly, the authors found a slight decrease in white matter injury, striatal and hippocampal tissue loss at 10 wk after HI in female rats administered the lower tested dose, but more importantly, they found that EPO improved sensorimotor function at 4 and 9 wk after injury in this female group.

The gender-specific neuroprotective effects described in this study are consistent with an earlier study of EPO (9) and have also been suggested in studies of 2-iminobiotin (10) and hypothermia (11). Although the authors propose that this female-only EPO response may be secondary to increased frequency of certain EPOR alleles in females, the mechanisms remain largely unknown. Sexual dimorphism in brain development is typified by surges in circulating hormones present during prepubertal and pubertal stages, as well as differential gene expression at the cellular level observed even in the absence of hormonal influence (12). Against this backdrop, gender differences in brain injury and response to therapies such as EPO may not be surprising. Indeed, gender-specific patterns of gene expression were recently described in a microarray study of neonatal HI and EPO therapy (13). Such differential gene expression may explain the authors' observation of increased hippocampal precursor proliferation after EPO administration, indicating gender-specific enhancement of neurogenesis, which has thus far only been described in the adult rodent (14).

It is worth noting that the beneficial effects of EPO in this study were not only gender-specific but also dose-specific. Although no consensus exists for optimal EPO dosage, timing, and frequency, EPO has been shown to have beneficial effects

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when used with several different treatment strategies (3,15). Consistent with these studies, the authors of this study demonstrated EPO neuroprotection at a dosage 5 kU/kg, but not 20 kU/kg. The dosing regimen used in this study was relatively brief, compared with recent studies that suggest more prolonged EPO administration may improve long-term behavioral effects (3,8,16). This may explain the relative lack of long-term histological improvement. Further clarification of EPO's mechanisms of repair will be crucial to determine the optimal dosage regimen that maximizes beneficial outcomes.

The importance of examining both the histological and behavioral effects at early and late time points cannot be understated. Although this study did examine effects of EPO therapy after neonatal mouse HI at early and later time points, the behavioral testing was limited to sensorimotor function and did not assess cognitive function or outcomes that persisted into adulthood. To clarify optimal treatment protocols of EPO, including dose amount, number, and timing, will involve thorough evaluation of long-term histology and both sensorimotor and cognitive function. This will provide a basis for further testing of neuroprotective or neuroreparative therapies in the future.

Although EPO monotherapy shows promise, single therapy after brain injury may only result in limited improvement (17). Early studies in human neonates with HIE demonstrate safety and benefit, with improved neurological outcome at 18 mo of age (18,19). In adult studies, EPO improved short-term outcomes after ischemic stroke (20), but more recent studies have raised concern for adverse effects, such as thrombosis (21). Hypothermia has become the standard of care in many institutions; however, it does not completely protect or repair an injured brain, and benefits may not necessarily be long-lasting (22). Combination therapy may provide more long-lasting neuroprotection, salvaging the brain from severe injury while also enhancing repair and regeneration. For example, therapeutics targeting apoptosis may prevent delayed cell death but would not affect earlier necrotic and excitotoxic injury. Combination therapy may also provide a window for protection if hypothermia is delayed, which is possible given difficulty in initiation of cooling if infants are born at an outside hospital or transport is delayed. However, care must be taken to avoid therapies that may adversely affect growth and development of the newborn. Studies examining the safety and efficacy of EPO and hypothermia in both animal models and humans are ongoing. It will be important to continue to develop models to evaluate the vulnerability of the immature brain to these treatments and their effects on short- and long-term neurodevelopment.

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