Adult or Perinatal Brain Injury : Does Sex Matter?

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Biological sex and sex-defining steroids are strikingly under-rated as modulators of cerebral ischemic cell death. In adults, male sex has been long identified as a risk factor for clinical stroke, yet the biology behind this fact remains veiled. We know that overall stroke incidence is lower in women than in men, across widely varying ethnic and cultural backgrounds.[1] Newer glimpses into this sexual dimorphism indicate that women continue to sustain lower stroke rates until beyond the menopausal years, suggesting that hormonal factors are not solely responsible. For example, stroke rates in female subjects of the Northern Manhattan Stroke Study did not exceed those of men until aged ≥85 years.[2] Importantly, data from sex-stratified preclinical studies indicate that stroke sensitivity (the damage resulting when an ischemic insult occurs) is also sexually dimorphic in adults. It is less clear if ischemic injury in the developing brain develops differently in males and females. However, provocative new evidence from cells cultured directly from fetal or newborn brain suggests that mechanisms of cell death are not identical in cells that are genetically male (XY) versus female (XX). This article evaluates linkages between sex, sex steroids, and neuroprotection throughout life.

Adult Brain Injury Is Sexually Dimorphic

The presence of a male "ischemia-sensitive" phenotype has been suggested in a wide variety of animal studies. One of the most impressive early studies included >2000 animals.[3] Yamori et al showed that life expectancy was longer in female spontaneously hypertensive stroke-prone rats, and the development of cerebral hemorrhage and vascular lesions was attenuated relative to male spontaneously hypertensive stroke-prone rats.[3] Subsequently, female rats and mice of numerous inbred and outbred strains have been studied. These studies clearly demonstrate less tissue damage for an equivalent insult after focal or global cerebral ischemia in age-matched females versus male,[4–6] accompanied by improved functional outcome.[7] Similarly, female animals sustain less damage after concussive brain injury and, unlike males, do not benefit from hypothermia as a therapy.[8–9] Complex experimental stroke models have been accomplished in animals with genetic stroke risk factors: insulin-dependent genetic diabetes,[10] non-insulin–dependent diabetes,[11] and hypertension.[5] In each of these studies, males are more sensitive to cerebral ischemia than are females.

Without doubt, sex steroids provide an infrastructure on which ischemic cell death is played out. The principal estrogen, 17β estradiol, has been widely shown to reduce neuronal death in vivo and in vitro and to stabilize preischemic vascular performance. The list of mechanisms, proven or putative, by which estradiol provides protection is long.[12] Accumulating data also suggest that progesterone protects brain from ischemic or traumatic injury, acting as an anti-edema agent.[13–14] The effect of androgens in ischemic injury is largely unknown. Available data with testosterone suggest deleterious effects in the intact brain exposed to ischemia[15] but protective actions in vitro.[16]

Sex-specific sensitivity to cerebral ischemia may also be because of differences in utilization of molecular cell death pathways by males and females. Data from genetically engineered mice support this hypothesis when both sexes are studied. Ischemic outcome in knockout mice can be overtly gender-dependent, even in strains where the gene of interest is not linked to reproduction or sexual development. Examples include the inducible[17,18] and neuronal isoforms of nitric oxide synthase (NOS)[19,20] and the DNA repair enzyme poly (ADP-ribose) polymerase (PARP-1).[20] It is now well accepted that NO generated during ischemia leads to neuronal death in part from its rapid reaction with superoxide anion, leading to peroxynitrite formation and protein nitration.[21,22] Key evidence that helped to establish the NO hypothesis arose from studies in male animals where genetic deletion or pharmacological neuronal NOS inhibition reduced ischemic damage. New observations now show that female nNOS knockout mice, or wild-type females treated with well-studied enzyme inhibitors, sustain paradoxically–increased damage after experimental stroke. Further, the paradoxical response in females is not explained by a protective action of estradiol.[20] Another well established cell death mechanism involves PARP-1 activation after DNA damage emerging from excitotoxicity or ischemia.[23] Data obtained from male PARP-1 knockout mice or from mixed sex neuronal cultures emphasize that halting PARP-1 activation improves cell

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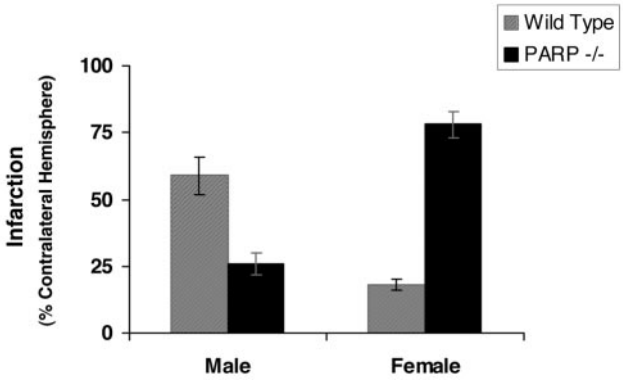

Figure 1. Genetic deletion of poly (ADP-ribose) polymerase (PARP-1) produces sex-dependent outcome after middle cerebral artery occlusion. Damage was determined by standard histology in male and female PARP-1 knockout mice and wild-type littermates. Data source, reference.[20]

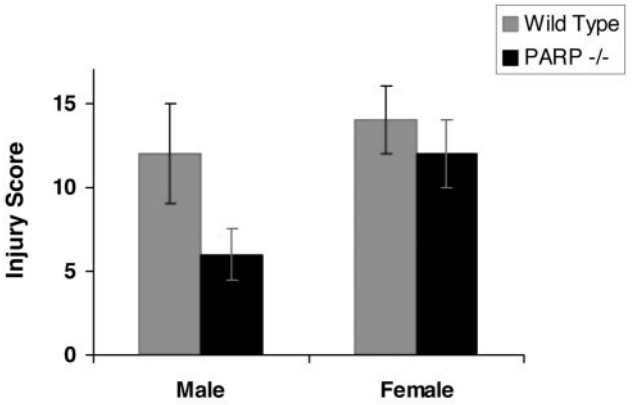

Figure 2. Genetic deletion of PARP-1 produces sex dependent outcome following hypoxia-ischemia in postnatal mice. Total injury score was determined by standard cresyl violet histology (10 sections per animal) in 7 day old male and female PARP-1 knockout mice and wild-type littermates.[37]

recovery.[24–25] However, loss of PARP-1 activity in female knockouts, or in wild-type females treated with specific PARP inhibitors, hugely exacerbates ischemic damage (Figure 1).[20] Although it is not clear how these cell death pathways diverge in the male and female, these data suggest that sex can alter the molecular context of brain injury.

Sex and Perinatal Injury

The role of sex in determining an "ischemia-sensitive" phenotype in the pediatric population is far less clear. A study for risk factors for stroke among children with sickle cell disease found no effect of sex on stroke prevalence,[26] nor was there an effect of gender on mortality after pediatric traumatic brain injury.[27] However, after pediatric traumatic brain injury, girls demonstrate a significantly better outcome in tests of learning and memory.[28–29]

In experimental animal models, reduced sensitivity to ischemic injury in females has been attributed to circulating estradiol, a factor not relevant to prepubertal animals. Therefore, it has been customary in studies of neonatal ischemic brain injury to evaluate hypotheses without sex stratification. Limited data in sex-specific protocols are available. Assessment of long-term neuropathologic outcome after unilateral cerebral hypoxia-ischemia (HI) in the immature rat (postnatal day, P7) demonstrated that the severity of damage was linearly linked to the duration of HI.[30–32] No difference in outcome was observed between male and female pups. More recently, decreased vulnerability to HI has been reported in females relative to their male littermates, and the sexual dimorphism emerges between P21 and P60.[33] This timeframe is likely coincident with the onset of sexual maturation and sex steroid production.

However, sexual dimorphism in central nervous system development is apparent during both embryonic and postnatal periods preceding puberty. Differences in gene expression are evident in males versus females by midgestation in the rodent,[34] and surges of sex steroids during late embryonic and early postnatal life program the brain.[35] Furthermore, even if the extent of brain damage does not differ between male and female postnatal rodents, emerging evidence suggests differences in the mechanisms leading to cell death. For example, post-HI hypothermia provides long-term protection in neonatal rats in a sex-specific manner.[36] Reduction of lesion size is quite limited in males, without significant functional improvement, whereas hypothermia provides robust protection in female rats with significant reduction in sensory-motor deficits (total functional rank 100 ± 34 in females versus 150 ± 35 in males). A recent study demonstrates that a similar gender-dependent response to PARP deletion seen in the adult is also present in the neonate, in that postnatal female and male mice subjected to HI responded differently to PARP-1 gene deficiency.[37] Male PARP-1 knockouts enjoyed a $\approx$50% reduction in histological damage, whereas injury in postnatal females was unaffected by the gene dose of PARP-1. PARP-1 activation, as measured by poly-ADP ribose accumulation, was not different between the sexes (Figure 2). However, PARP-1 uses NAD^+ to ribosylate DNA, a scarce commodity during ischemia and energy failure. NAD^+ reduction was more pronounced in male versus female pups, suggesting differences at the mitochondrial level.

"Sex-ed" Cells and In vitro Ischemic Sensitivity

Further support that cell death mechanisms can be sex-specific arises from studies that use cell cultures in which background sex steroid exposure is removed. This approach allows an evaluation of the hypothesis that cellular response to a molecular insult diverge, depending on the genetic sex of the cell (XX or XY). Female dopaminergic neurons (embryonic day 14 cultures, E14) tolerate exposure to toxic dopamine concentrations and survive 2-fold relative to male cells.[38] Similarly, female neurons (E19) cultured from cortical plate or ventricular zone have greater longevity than do male cells and differentially express higher levels of phosphorylated kinases, such as Akt.[39] Sensitivity to glutamate, peroxynitrite (ONOO), and staurosporine in neuronal culture (E17) has recently been reported to be sex specific, with male neurons being more susceptible to glutamate and ONOO than females. In contrast, oxidants, such as H_2O_2, damage both sexes equally.[40] Sexually dimorphic responses also occur in astrocyte cultures (rat P3) where death after oxygen-glucose deprivation is greater in male cells than in female cells.[41]

Conclusions

These studies represent a very early understanding of the role of biological sex and sex-defining steroids on ischemic pathobiology in the adult or immature brain. Emerging evidence suggests that some cell death pathways are sex specific; the breadth of these observations and underlying mechanisms remains to be assessed. The use of both sexes in preclinical animal experiments and in cell cultures stratified by sex will continue to provide useful information. If so, then gender-based therapeutic interventions may well hold promise for greater neuroprotection in adult and perinatal brain injury.

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