Sex Differences in miRNA Expression in Hippocampus of Neonatal Piglets after Isoflurane Anesthesia: A Pilot Study

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Background
A large body of preclinical evidence in animal models has raised significant concerns that anesthesia exposure in early life may lead to neurocognitive deficits later in life. It has become incumbent upon the pediatric anesthesia community to further understand and elucidate the molecular mechanisms that may be responsible for anesthesia-induced developmental neurotoxicity (AIDN) in children. Despite the obvious effect of sex on the pathogenesis of multiple neurologic diseases, very little research has focused on the effect of sex on response to anesthesia. This study examined the effects of anesthesia on miRNA expression. miRNAs are small, non-coding RNAs that are involved in post-transcriptional RNA modification/regulation. They have been shown to be involved in numerous neurodegenerative processes. We targeted miRNAs as a potential mechanism of AIDN. This work addresses four key factors: 1) the novel use of a translational piglet model to study AIDN; 2) miRNAs as tools to measure changes in neurochemistry in response to anesthesia, 3) modulation of miRNA expression in response to anesthesia, and 4) the effect of sex on modulation of miRNA expression.

Methods
• Piglets (6M, 6F), aged 3-5 days, were anesthetized as previously described1
• Anesthesia was maintained with 2.5% isoflurane in room air for 3 hours (clinically relevant exposure)
• Piglets received full physiologic monitoring equivalent to what is used in children at all times
• After exposure, the animals were sacrificed and the hippocampus was removed and flash-frozen.
• Untreated animals (6 male, 6 female) served as controls
• Samples from isoflurane-treated animals and controls were analyzed
• Relative concentrations of miRNAs via a GeneChip Porcine miRNA 4.0 Array (measures 326 miRNAs) were assessed

Results
• The following groups were analyzed:
  • Male vs. female control
  • Male control vs. male iso
  • Female control vs. female iso
  • Striking similarity in expression patterns between male and female controls (Figure 1)
  • M vs. F Control: 17/326 (5%) miRNAs significantly dysregulated (P<0.05)
  • Male control vs. Male iso: 17/326 (5%) miRNAs dysregulated (P<0.05)
  • Female Control vs. Female iso: 14/326 (4%) miRNAs dysregulated (P<0.05)
  • 5 dysregulated miRNAs were common to the MvF control and male control vs. iso groups, while only 1 was common to the MvF control and female control vs. is group (Figure 2)

Conclusions
• In neonatal piglets, miRNA expression is highly conserved when male and female animals are compared
• Our data suggest that sex differences in isoflurane-induced miRNA dysregulation could be due, in part, to physiologic differences between males and females
• Isoflurane-induced miRNA dysregulation is a sex-dependent phenomenon, yielding distinct expression profiles in males and females, which suggests distinct mechanisms of dysregulation
• Increased overlap between groups in male animals leads to the hypothesis that virilizing hormones (eg testosterone) may be involved in sex-specific differences in anesthesia-modulated miRNA dysregulation
• A significant number of dysregulated miRNAs are involved in critical functions such as cell-cycle arrest and apoptosis, making them excellent targets for further study

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References