Exposure to inhaled anesthesia does not induce neuronal apoptosis in neonatal piglets

EE Whitaker, 1 JK Lee, 1 AG Larson, 1 JL Jamrogowicz, 1 E Kulikowicz, RC Koehler, 1 LJ Martin 2

1Dept. Anesthesiology/Critical Care Medicine, Johns Hopkins Univ. (JHU), Baltimore, MD. 2Dept. Pathology, Johns Hopkins Univ. (JHU), Baltimore, MD

Abstract

Background: Millions of children receive anesthesia each year worldwide. Studies in immature rodents and non-human primates suggest that exposure to inhaled anesthesia may have deleterious effects on the developing brain. Prior studies have relied upon detection of activated caspase-3 as a marker for presumed apoptosis without evidenced based on morphology or DNA fragmentation. (1) This method may overestimate the degree of neurotoxicity because caspase-3 is involved in non-apoptotic signaling. (2) Complete resolution of neuronal apoptosis has not been consistently demonstrated after inhaled anesthesia. Our objective was to evaluate neurotoxicity measured by apoptotic profile presence in neonatal piglets exposed to isoflurane or isoflurane + nitrous oxide (N2O). Methods: Neonatal piglets (2-3 days old) were exposed to 6 h of isoflurane 2% (n=6) or isoflurane 1.5% + 30% N2O (n=7), recovered for 2 days, and euthanized. Naïve (untreated) age-matched piglets were controls (n=3). The piglets were transcardially perfused and the brains were removed for histologic examination with hematoxylin and eosin staining. Coronal sections were matched for anterior/posterior level as determined by anatomical regions. Apoptotic profiles, defined by nuclear and cytoplasmic features, were counted in CA1 of the anterior and posterior hippocampus and in 2 levels of piriform cortex in cortical layer 2 at 400X by a single investigator who was blinded to treatment group. Counts in piriform cortex were averaged between the 2 levels for analysis. Data were analyzed by one-way ANOVA.

Results: In all treatment groups, very few end-stage apoptotic profiles were identified. No difference was found in the number of apoptotic profiles in anterior hippocampus (p = 0.219), posterior hippocampus (p = 0.984), and piriform cortex (p = 0.467) among treatment groups. (Table 1) No difference was found in the number of apoptotic profiles in anterior hippocampus (p = 0.984), posterior hippocampus (p = 0.467), and piriform cortex (p = 0.467) among treatment groups. (Table 1)

Discussion: In this piglet model, few apoptotic profiles were identified in the hippocampus and piriform cortex after exposure to isoflurane or isoflurane + N2O and 2 days of recovery. The anesthesia exposure duration and anesthesia concentrations in this study are similar to previous studies, (3) and relevant to clinical practice. Our preliminary work suggests that, in neonatal piglets, inhaled anesthesia does not result in acute irreversible neurotoxicity characterized by apoptosis. Additional studies that investigate, in immature brain, regional total neuron numbers, non-apoptotic caspase-3 functions, and non-apoptotic cell death activation after inhaled anesthesia are indicated.

Hypothesis

• Exposure to isoflurane and N2O will result in more neuronal apoptosis than exposure to isoflurane alone, or in naïve controls

Methods

• Neonatal male piglets (age 2-3 days old) were anesthetized with 5% isoflurane and a 70/30% mixture of N2O in oxygen via a face cone

• Once adequate anesthesia was attained, the piglets were intubated with a standard laryngoscope

• After the prep and the femoral artery and vein were cannulated for medication administration, blood sample analysis, and direct arterial pressure measurement.

• Piglets remained anesthetized for 6 hours, receiving 2% isoflurane or 1.5% isoflurane + 70% N2O in 30% oxygen.

• While apoptotic profiles were seen in treatment groups, there were very few (Figure 1).

Conclusions

• Though concentration and duration of exposure were clinically relevant, our study did not reveal significant apoptosis, leading us to conclude that this anesthetic regimen does not cause neuronal apoptosis in neonatal swine

References

