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Summary

All commonly used general anesthetics have been shown to cause neurotoxicity in animal models, including non-human primates. Opinion, however, remains divided over how cumulative evidence from preclinical and human studies in this field should be interpreted and its translation to current practices in pediatric anesthesia and surgery. A group of international experts in laboratory and clinical sciences recently convened in Genoa, Italy, to evaluate the current state of both laboratory and clinical research and discuss future directions for basic, translational, and clinical studies in this field. This paper describes those discussions and conclusions. A central goal identified was the importance of continuing to pursue laboratory research efforts to better understand the biological pathways underlying anesthesia neurotoxicity. The distinction between basic and translational experimental designs in this field was highlighted, and it was acknowledged that it will be important for future animal research to try to causally link structural changes with long-term cognitive abnormalities. While inherent limitations will continue to affect the ability of even large observational cohorts to determine if anesthesia impacts neurodevelopment or behavioral outcomes, the importance of conducting further large well-designed cohort studies was also emphasized. Adequately powered cohorts could clarify which populations are at increased risk, provide information on environmental and healthcare-related risk modifiers, and guide future interventional trials. If anesthetics cause structural or functional adverse neurological effects in young children, alternative or mitigating strategies need to be considered. While protective or mitigating strategies have been repeatedly studied in animals, there are currently no human data to support alternative anesthetic strategies in clinical practice. Lastly, it was noted that there is still considerable debate over the clinical relevance of anesthesia neurotoxicity, and the need to evaluate the impact of other aspects of perioperative care on neurodevelopment must also be considered.

Key words: pediatrics, anesthesia, neurodevelopment, neurotoxicity, research, clinical trial
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Introduction

The impact of anesthetic exposure on the developing brain continues to be a topic of extensive debate and ongoing research. Over 500 preclinical studies of anesthesia neurotoxicity have been

published, and structural abnormalities and functional changes have been demonstrated in a variety of animal models, including non-human primates.[1] These adverse effects are seen with all commonly used general anesthetics. Accordingly, substantial concerns have been raised for the safety of young children undergoing repeated or prolonged anesthetics, culminating in safety warnings issued by the US Food and Drug Administration.[2,3] Expert opinion, however, remains divided over the applicability of preclinical studies to humans, the interpretation of current human studies, and how these findings should influence clinical practice.[4] Given the potential impact on the millions of children who require surgery each year and the uncertainty over current evidence, further high-quality multidisciplinary research is required to clarify and, if necessary, mitigate risks of anesthesia neurotoxicity.

A group of international experts in laboratory and clinical sciences in this field convened in Genoa, Italy, from May 13-14th, 2017 for the *2nd International Conference on Pediatric Anesthesia and Neurotoxicity: From the GAS Study to future collaborative trials*. The workshop was partly funded by a grant from the Italian Ministry of Health to support the GAS Study (RF-2011-02347532). Twenty-eight experts formed the faculty and an additional 35 delegates attended the 2-day workshop. The workshop built upon a previous one held with a similar mandate in 2014.[5] During the workshop, the current state of both laboratory and clinical research was evaluated and future directions for new basic, translational, and clinical studies were discussed. This meeting report does not aim to be a comprehensive review of the topic but summarizes the key discussion points arising from the workshop.

Current state and future directions for preclinical studies

Animal studies have repeatedly demonstrated that exposures to all commonly used general anesthetics, albeit often on the upper boundaries for durations and doses seen in clinical practice, can lead to alterations in trophic factors, synaptic and dendritic architecture, and widespread neuronal cell loss.[1] Several preclinical studies have also found a range of long-term neurodevelopmental changes in animals exposed to general anesthetics in early life, especially in domains of memory and learning, but also changes in some aspects of behavior. Given the pervasive effects of anesthetics on signaling pathways and cellular homeostasis in the central nervous system, the underlying mechanisms of injury are only incompletely understood.[6]

A central goal identified by workshop discussants was the importance of continuing to pursue laboratory research efforts to better understand the biological pathways underlying

anesthesia neurotoxicity. It is imperative to further elucidate the phenomenon's underlying mechanisms to better define human applicability, identify safer anesthetic techniques, and devise mitigating strategies. Since no biological tenet exempts humans from the structural and functional abnormalities observed in animals, preclinical studies offer key opportunities for future research efforts.

Both *in vitro* and *in vivo* models are required to elucidate mechanisms of injury at the molecular, cellular, and systems levels, and the primary goal of this *basic* science research activity should be to identify specific molecular targets and neural pathways involved in anesthetic neurotoxicity. This preclinical platform would, in turn, support focused theory generation and model development, providing a driving force behind translational science. The distinction between basic and translational science is important to recognize in this field, as they result in significant differences in experimental designs. It is important to emphasize that ***basic science*** is primarily an exploratory research activity where every step is highly dependent on the observations made in the previous step of the same experimental process. Therefore, as experiments of anesthesia neurotoxicity involve stepwise interdependent explorations of crossing molecular pathways, working hypotheses, experimental endpoints, and power calculations can be difficult to define in advance. This is in striking contrast with ***translational or applied science***, which refers to the applicability of basic scientific discoveries to real-world problems.[7]

In terms of translational relevance, studies using non-human primates are important in the experimental field of developmental anesthesia neurotoxicity. Non-human primates more closely resemble human physiology and pathophysiology than any other animal species, especially during pregnancy and early development. [8] Behavioral studies have also shown that well-trained non-human primates can perform certain tasks with comparable accuracy to children, supporting their extrapolation to humans. [9] When using small animal models, monitoring of vital signs and maintenance of homeostasis are critical during anesthesia exposure to reduce risk of confounding. When elucidating the mechanisms underlying long-term effects of anesthetic exposures on neurodevelopment, it will also be important for future animal research to causally link structural changes observed in immature brains immediately following exposure with long-term cognitive abnormalities. While acknowledging that this may be difficult, discussants expressed optimism that this goal could be reached by exploiting serum or imaging biomarkers. In general, it was acknowledged that future experimental studies will require substantial improvements in quality and reproducibility and should adhere to the appropriate conduct and reporting guidelines (e.g.,

ARRIVE [**A**nimal **R**esearch: **R**eporting of ***I**n **V**ivo* **E**xperiments]) guidelines.[10]

Recent clinical studies

The GAS (**G**eneral **A**nesthesia *vs*. **S**pinal) randomized controlled trial and several cohort studies have recently provided important new evidence. The GAS trial randomized infants undergoing inguinal hernia repair to a sevoflurane-based general anesthetic or a neuraxial block without sedation. In this study, the average exposure to anesthesia was just under an hour in length, and the trial found good evidence for no difference in the secondary outcome of neurodevelopment at 2 years of age measured with the Bayley III Scales of Infant and Toddler Development. [11] The primary outcome, neurodevelopment at 5 years of age, will be known in 2018. In addition, the PANDA (**P**ediatric **A**nesthesia & **N**euro**D**evelopment **A**ssessment) study found no differences across multiple neuropsychological and behavioral measures in later childhood for children who had a single anesthetic exposure for inguinal hernia repair prior to 3 years of age compared with unexposed siblings. [12]

In addition, several recent cohort studies have found modest evidence for an association between general anesthesia exposure and surgery in early childhood and later abnormal behavioral, learning, and neurodevelopmental outcomes. Findings from two Canadian Provincial (Manitoba and Ontario) cohorts, which both used the Early Development Instrument (a teacher administered assessment of children's readiness to learn at school entry), suggest there is a small increase in risk of adverse child development after anesthesia and surgery.[13,14] Similarly, a Swedish population-based study demonstrated slightly lower school grades (at 16 years of age) for children who were exposed to anesthesia and surgery prior to 4 years of age compared with unexposed children.[15] Interestingly, in the study by Graham *et al.* there was good evidence that adverse effects were greater in children exposed at an older age, and none of these three studies found evidence of increased risk with multiple exposures to anesthesia compared to single exposure. While these cohort studies only found small effect sizes, given the prevalent use of general anesthesia in young children for both surgical and non-surgical procedures, these findings may nevertheless be significant from a societal perspective. Similarly, even a small increase in the incidence of significant neurodevelopmental disabilities is important at a population level.

Other earlier observational studies in humans also report associations between anesthesia exposure in early childhood and increased risk of adverse behavioral and educational outcomes. [16] Of note, another recent cohort study has also reproduced the finding of an earlier

study cohort from Olmsted County, Minnesota, finding that there is a an association between exposure to anesthesia and surgery in young children and increased risk of diagnosis of Attention-deficit/Hyperactivity Disorder (ADHD) or learning disabilities.[17] In these instances, the association was stronger after multiple exposures. However, like all observational studies, these findings must be considered in the context of possible confounding (e.g., familial and genetic contributions to ADHD and increased healthcare utilization) and it is difficult to suggest a causal relationship between anesthesia exposure and ADHD from these studies. [18]

Future observational studies

While the risk of unmeasured confounding factors and the inability to infer causality between exposure and outcome continue to limit the ability of even large observational cohorts with rich sources of data to determine if anesthesia *per se* has any impact on neurodevelopment or behavioral outcomes, the importance of conducting further large well-designed cohort studies was emphasized by the discussants. Future epidemiological studies are needed to further clarify which children are at increased risk, what outcome domains are affected, and whether a specific anesthesia neurotoxicity phenotype can be identified. If sufficiently powered across age groups, such studies may also help to determine whether a specific developmental window of vulnerability exists. They may also provide information about the role of potential environmental and healthcare-related (e.g., surgery-specific factors) risk modifiers. They will also be essential to guide which populations should be included in any future interventional trials. However, epidemiological studies need to be large enough to detect small differences between groups and be able to collect appropriate data for each included child (i.e., healthcare, social, home environment, comorbidities, and perioperative factors). Since currently available clinical data suggest that a brief single exposure in healthy children might not increase the risk of poor neurodevelopmental outcome, future cohort studies should focus on children with specific comorbidities and those undergoing multiple or prolonged anesthetic exposures. While cohort studies can never eliminate significant risk of confounding, appropriate sampling and rigorous analysis may reduce this impact.

The Mayo Anesthesia Safety in Kids (MASK) is a prominent example of a cohort study which is expected to report its initial results shortly.[19] This propensity-matched sample of nearly 1000 children from a birth cohort in Olmsted County, Minnesota identified children's exposure to anesthesia (none, single, or multiple) before 3 years of age, and then had a comprehensive range

of direct neurocognitive tests and behavioral assessments performed after 8 years of age.

Similar to preclinical studies, future observational studies need to adhere to the appropriate reporting guidelines (e.g. RECORD [**RE**porting of studies **C**onducted using **O**bservational **R**outinely-collected **D**ata] guidelines for using health data), [20,21] and follow recent guidelines for the a-priori publication of analysis plans to enhance the trust, validity, consistency and confidence in data collection and reporting. [22]

Determining appropriate outcome measures in clinical studies

There is still debate over which neurological, neuropsychological or neurobehavioral domains should be tested and at which ages, and current recommendations may change as more preclinical and clinical data emerge. Accordingly, ongoing close collaboration with neuropsychology experts will be essential to devise appropriate testing strategies. Testing at older ages allows assessment of a wider range of neuropsychological domains and tests often have greater predictive power for neurological function into adulthood.[23] Moreover, an extended period between exposure and testing may increase the likelihood of the consequences of any injury becoming apparent; for example an injury causing a deficit in memory or learning will not immediately be reflected in reduced IQ. However, this longer time interval has practical implications for the dissemination and translation of research findings, and conducting these studies becomes more difficult as longer intervals to testing may result in greater loss to follow-up.

Currently, only limited outcomes are available from secondary use of existing databases (i.e., clinical, educational, demographic) and routinely collected personal medical records. Improvements in electronic medical records, data-linkage capacity, and privacy regulations may present greater opportunities for their use in clinical research of anesthetic neurotoxicity. However, as noted before, available outcomes may have limited sensitivity to detect deficits in specific domains of neurodevelopment. [24]

Future trials

Randomized controlled trials remain the gold-standard study design for determining causality, but they can have significant limitations. When studying anesthesia neurotoxicity, difficulties include determining the best patient populations, interventions and mitigation strategies to test, finding an appropriate comparator group, enrolling sufficient children,

significant cost, and the lag time between testing interventions and measuring outcomes. Large sample sizes for these trials will also be needed to detect or exclude clinically relevant differences. The cost and long study period of clinical trials underscores the potential utility of using surrogate outcomes, such as biomarkers. Similarly, neuro-imaging could provide short-term outcomes by assessing brain structural and functional integrity. Imaging could also provide mechanistic insight if imaging changes can be correlated with long-term functional outcomes.

A new multicenter randomized controlled trial, the TREX (**T**rial **R**emifentanil and d**EX**medetomidine) trial, designed to test a mitigation strategy for anesthesia neurotoxicity has now started enrolment in North America, Europe and Australia. It randomizes infants and toddlers less than 2 years of age, who are expected to undergo anesthetic exposures of 3 hours or longer to customary doses of sevoflurane or to a dexmedetomidine/remifentanil/low dose sevoflurane technique, and initial neurodevelopmental testing will be performed at 3 years of age.

Alternative and mitigating strategies

If anesthetic exposure causes structural or functional adverse neurological effects in young children, alternative or mitigating strategies need to be considered. Simply withholding anesthesia is unethical, and also untreated pain and distress in developing animals and young children is likely to cause harm. Moreover, it is currently unclear whether an age exists after which anesthetic neurotoxicity risks are lessened.[25] If childhood procedures are required, efforts should be made to limit the duration of exposure and/or the dose of anesthetic drugs. Further defining the optimal doses of anesthetic drugs would also benefit children, regardless of the discussion around anesthetic neurotoxicity. For example, a combination between regional anesthetic techniques and general anesthesia or deep sedation facilitate substantial reductions in the doses administered of potentially harmful anesthetic drugs.

Protective or mitigating strategies have been repeatedly studied in animals but not in humans. These studies have demonstrated that a wide variety of compounds or treatments, such as neurotrophic p75NTR or RhoA receptors, cytoskeletal stabilizers jasplakinolide or TAT-Pep5, antioxidants resveratrol, vitamin C, melatonin, L-carnitine, arachidonic acid, β-estradiol, lithium, carbon monoxide, hypothermia, xenon, dexmedetomidine, painful stimulation, as well as isoflurane preconditioning may all afford some degree of protection from structural abnormalities or functional impairment observed during anesthetic exposure. However, the safety of most of these modalities has not been tested in clinical studies and, at this stage, they cannot be

recommended as protective or mitigating strategies. Most notably, some preclinical studies suggest that opioids or alpha-2 adrenergic receptor agonists may be less toxic than commonly used anesthetic drugs. However, the results of these preclinical data are inconsistent. For example, several studies have confirmed only lesser toxicity of dexmedetomidine compared with sevoflurane,[26] while others showed various degree of protection provided by dexmedetomidine when co-administered with an injurious dose of sevoflurane.[27] Importantly, currently, no clinical data exist to support alternative anesthetic strategies.

Conclusions

In summary, some of the key aspects identified for future research in this field were:

- The need for high-quality preclinical studies, both basic and translational, to evaluate mechanisms of toxicity and to inform choice of anesthetic techniques and/or mitigating strategies.
- Cohort studies with rich sources of data and large enough to detect small outcome differences are required to better characterize neurodevelopmental domains potentially affected in children, identify vulnerable populations, and to establish clinical risk modifiers.
- Carefully designed and adequately powered clinical trials testing plausible interventions in relevant patient populations are required for translation of research to clinical practice.

It was emphasized that the planning of future studies should encourage close collaboration between preclinical scientists, neuropsychologists, neonatologists and developmental pediatricians, neurologists, toxicologists, epidemiologists and the anesthesia community. Further, It was noted that there is still considerable debate over the clinical relevance of anesthesia neurotoxicity, and the need to evaluate the impact of other aspects of perioperative care on neurodevelopment must be considered.

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