Nitrous oxide ($N_2O$) toxicity and cobalamin ($B_{12}$)-dependent metabolism

Kirk Hogan M.D., J.D.
<khogan@wisc.edu>
Department of Anesthesiology, University of Wisconsin – Madison
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No conflicts
History of N₂O use

- 1810s - First recognition of N₂O analgesic properties
- 1840s - First demonstration of N₂O anesthetic properties
- 20th century - N₂O “balanced” with inhaled volatile agents to reduce toxicity and side effects
- 1938 - Food, Drug and Cosmetic Act
- § 201.161 Medical gases (April 1, 2017).
- “(a) Oxygen, nitrogen, carbon dioxide, helium, and nitrous oxide gases intended for drug use, and medically appropriate combinations of any of these gases intended for drug use, are exempted from the requirements of § 201.100(b)(2) and (3), and (c)(1)"
- “(c)(1): (1) Labeling on or within the package from which the drug is to be dispensed bears adequate information for its use, including indications, effects, dosages, routes, methods, and frequency and duration of administration, and any relevant hazards, contraindications, side effects, and precautions under which practitioners licensed by law to administer the drug can use the drug safely and for the purposes for which it is intended, including all purposes for which it is advertised or represented;”
- The FDA has not investigated or established the safety and efficacy of N₂O
- Ask your colleagues and patients if they know this
History of N₂O toxicity

• Late 1940s - N₂O uniformly lethal in treatment of tetanus pain in post-war Europe
• 1970s - **Subacute** combined degeneration of the spinal cord after **recreational use**
• 1990s – **Acute** combined degeneration of the spinal cord after **clinical use** in standard concentrations and durations (e-mail for reference list)
• 1990s – N₂O NIOSH and OSHA regulated at **8 ppm** vs. FDA (and DEA) **unregulated** at **700,000 ppm** +
• 2000 - **Acquired predisposition** to N₂O neurotoxicity (Felmet *et al.*)
• 2003 - **Genetic predisposition** to N₂O neurotoxicity (Selzer *et al.*)
• N₂O is the only inhaled anesthetic that will kill everyone who breathes it at clinical concentrations for 5-7 days
• Issues are individual susceptibility, duration and concentration of use, cellular reserves of methionine, and how much damage is acceptable
THF
5,10-
Methylene
THF
5-Methyl
THF
5,10-Methylene
THF

MTHFR
MTR B₁₂
MTR-Co(III)
MTR-Co(II)
MTRR

CBS
Cystathionine

Homocysteine

S-Adenosyl
homocysteine
S-Adenosyl
methionine
Methionine
N₂O

Methyl
Donation

S-Adenosyl
homocysteine

Methionine

S-Adenosyl
methionine

Methyl
Donation

Methyl
Donation

Methyl
Donation

Methyl
Donation
N₂O Inactivation of Methionine Synthase

- N₂O inactivates methionine synthase in everyone
- Mean half time of human hepatic methionine synthase inactivation - 45 minutes – CNS unknown
- Recovery > 4 days to resynthesize enzyme i.e., not when nitrous oxide is DCed
- Serial exposure particularly harmful
  - Human fetal/neonatal and geriatric kinetics of inactivation and reactivation are unknown in any tissue
  - N₂O inhibition of methionine synthase is rapid, potent, and irreversible in all patients, and harmful in a substantial proportion of patients that are not identified before exposure
- Deleterious consequences are long-lasting, not “short acting”
- N₂O’s toxicity is distinct from N₂O’s site(s) of anesthetic action
Prevalent *MTHFR* Mutations

- MTHFR activity is reduced > 70% with common SNPs
- *MTHFR (C677T)* (Hogan et al. *Clin Med Res* 2009;7:69)
  - Homozygote – 13%
- *MTHFR (A1298C)*
  - Homozygote – 9%
- No compound homozygotes – lethal?
- % varies by geographic origin of ancestors
- Additional polymorphisms in intrinsic and extrinsic single carbon pathways
B-vitamin deficiency

• “The prevalence of vitamin B12 deficiency, whether defined as low vitamin B12 or metabolically significant vitamin B12 deficiency increased with age in all three studies, from about 1 in 20 among people aged 65-74 years to 1 in 10 or even greater among people aged 75 years or greater. The prevalence of folate deficiency also increased with age, and was similar to that for vitamin B12 deficiencies.”


• 23% incidence of B12 deficiency in third trimester and childhood


• Added to deficiencies in folate and B6

• Combined genetic and acquired susceptibilities in at least 5% of patients
Components of prospective study before and after N$_2$O exposure

- **B vitamin levels** in plasma, red blood cells and other cells
- Single carbon pathway **substrate and product levels** – homocysteine, methionine, etc.
- Single carbon pathway **genotypes and epi-genotypes (DNA methylation)**
- **Enzyme activities** in target tissues
- **Cellular indices** of bone marrow deficiency in circulating cells
- **Cellular indices of genotoxicity** *e.g.*, in cord blood, etc. (SCE, comet assay)
- **Clinical outcomes** – *e.g.*, neurotoxicity, marrow toxicity, other tissues, immediate and long term outcomes
- **Xenon** as a positive control
Informed consent:
The precautionary principle

- $\text{N}_2\text{O}$ has no established benefit in anesthetic maintenance compared to contemporary anesthetics
- $\text{N}_2\text{O}$ use is exploding outside the O.R. – e.g., labor and delivery, emergency rooms and ambulances, pain and psychiatry, pediatric procedural “sedation”, dental and oral surgery
- Patients are never tested for susceptibilities
- Patients and families are never informed of specific risks (ask for anesthesia informed consent law review in *Journal of Law and Medicine* (in press))
- $\text{N}_2\text{O}$ is a bone marrow gas
- $\text{N}_2\text{O}$ is a nerve gas
- The effects of $\text{N}_2\text{O}$ on regeneration of tissues after trauma and surgery have not been reported to date (ask to share submission-ready data)
If newly developed and introduced, could N₂O achieve FDA approval now?

Would a pharma executive wish to apply in view of minimal benefits and assured risks?

Would academic investigators wish to conduct appropriate prospective trials?

Would patients and parents of children wish to provide fully informed consent (i.e., “not FDA approved”) to participate in randomized trials antecedent to FDA approval?
Thanks!
Action Steps

• Must-read literature
• Clinical and research informed consent guidelines
• Research priorities
  • Labor & delivery
• Publication guidelines