Nitrous Oxide Genotoxicity

NITROUS oxide has long been known to be an impotent and unpredictable human anesthetic.\(^1\) In this issue of the journal, Chen et al.\(^2\) report that nitrous oxide in clinical use is a potent and predictable human genotoxin. The authors observe that maintenance of anesthesia with 70% nitrous oxide and sevoflurane in patients undergoing colorectal surgery doubles the incidence of DNA damage assessed by the in vitro alkaline single cell gel electrophoresis (i.e., “SCGE” or “comet”) assay compared or 80% oxygen and sevoflurane. Genotoxicity using the comet assay has been previously reported with chronic occupational exposure to low concentrations of nitrous oxide, after isoflurane anesthesia with and without nitrous oxide, and after sevoflurane anesthesia without nitrous oxide.\(^3-6\) The current clinical trial therefore fills an important gap with regard to anesthetics often used in combination. The author's observation that nitrous oxide-induced genotoxicity is associated with postoperative wound infection is original and warrants further consideration and confirmation.

The conventional alkaline comet assay detects DNA damage that occurs rapidly on exposure, and that may lead to fixed mutations in DNA including double- and single-strand DNA breaks, alkaline labile sites, DNA-protein and DNA–DNA cross-linking. On discontinuation of exposure, the primary lesions detected by the comet assay are most often correctly repaired in minutes to hours without persistent genetic alterations. Because it also detects single-strand breaks generated during the repair of initial damage including alkylated bases, bulky base adducts, and pyrimidine dimers, the comet assay provides an index of the kinetics of DNA strand break repair and break excision repair if serially performed. Although the kinetics and fidelity of DNA repair may vary with the type of lesion and tissue origin of target cells, little is known about interindividual differences in the capacity for DNA repair in humans.

Numerous attractive features of the comet assay have sustained its development and validation in the 30 yr since its introduction.\(^7,8\) As an “early warning system,” the comet assay detects very low levels of DNA damage (i.e., in \(1 \times 10^7\) base pairs), provides few negative results on exposure to known genotoxins and fewer false positive results than other cellular assays of genotoxicity including the micronucleus test, the chromosomal aberration test, and sister chromatid exchange. The comet assay is rapid, inexpensive and safe to perform, quantitative, generally free of artifacts, and is available in standardized kit formats that provide high interlaboratory replicability. Because low levels of DNA strand breaks and genetic instability detected by the comet assay after chemical exposures contribute to mutations and cancer in laboratory animals, its most prominent use at present is as a screening tool after in vivo exposure in research or regulatory test batteries.\(^7\)

The primary shortcoming of the comet assay is its uncertain predictive value in the absence of clear-cut and proven causal associations with specific human phenotypes. Because the clinical relevance of the comet assay’s primary endpoint, i.e., temporary strand breakage, is not known with precision, the conventional comet assay is not configured or validated to assess individual risk of disease phenotypes. Doubts about the comet assay’s analytical validity, clinical validity, and clinical utility have restrained widespread applications in medical and surgical settings. Given its many advantages, it is surprising that the comet assay has rarely been investigated in prospective cohort studies such as that described by Chen et al. Investigations designed to test for shared mechanisms of genotoxicity and deleterious outcomes, and to control for confounders such as reverse causality or association by chance alone, are fewer still. To further complicate the interpretation of comet assay results in humans, reports describing the

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effects on the comet assay of critical background variables such as gender, age, smoking, exercise, alcohol, and nutrition conflict with one another.

The reported association of nitrous oxide-induced comet assay abnormalities and wound infection is novel and provocative, albeit “exploratory” as the authors concede. Although the present data provide sample size estimates to assure that a follow-on study is amply powered, multiple opportunities to tease out causal mechanisms in human samples would be missed if increased enrollment is the sole design distinction between the present “hypothesis generating” protocol and a more definitive investigation. In addition to the DNA damage caused by folate deficiency hypothesized by the authors, comet assay abnormalities after nitrous oxide exposure may arise from direct homocysteine toxicity, oxidative damage, \( N \)-methyl-L-aspartate-mediated changes in lymphocytes and immune competent cells, or other mechanisms acting alone or in concert.\(^{10-12}\)

Accordingly, a first step in seeking mechanisms underlying genotoxicity as indexed by the comet assay, and its possible relation to wound infection after anesthetic maintenance with nitrous oxide, is to assure that sample cohorts are balanced for exposure to extrinsic genotoxins such as environmental and occupational pollutants (e.g., volatile organic hydrocarbons and organic solvents), pesticides, antineoplastic drugs, lead, metals, tobacco, and alcohol use, and for possible confounding variables including age, gender, exercise, diet, recent infection or inflammation, pregnancy, recent surgery, and parental geographic origin. As well, balancing for or excluding, participants with conditions known to cause hyperhomocysteinemia (e.g., folate and cobalamin nutritional deficiency, renal failure), inborn errors of single carbon metabolism, antifolate chemotherapeutics, and medications that increase homocysteine (e.g., oral hypoglycemics, anticonvulsants, levodopa, cyclosporine) is crucial. In addition to the baseline folate levels reported by Chen \textit{et al.}, prevalent single carbon pathway genotypes (e.g., in genes encoding methionine synthase \([\text{MTR}]\), \(5,10\) methylene tetrahydrofolate reductase \([\text{MTHFR}]\), and cystathionine \(\beta\)-synthase \([\text{CBS}]\)), and baseline and serial blood levels of homocysteine, methionine, cobalamin, pyridoxine, and related cofactors, substrates and products should be documented for all participants. An experimental design comprising nitrous oxide dose information that permits, for example, comparison between high and low quintiles of concentration and duration is to be strongly encouraged.

Chen \textit{et al.} reported comet assay data before and 24 h after surgery. To test for a parallel time course between DNA damage and wound infection, more frequent testing at 6, 12, and 24 h after surgery, and at 3, 5, and 7 days, or until the wound is healed would be of great value. In particular, the sensitivity and selectivity of serial comet assays may be improved by treating the slides with enzymes that convert specific DNA lesions to strand breaks such as endonuclease-III (Endo III) and formamidopyrimidine-DNA glycosylase to identify sites of oxidative damage, uracil glycosylase to detect uracil mis-incorporation, methylenadenine DNA glycosylase to identify \(3\)-methyl adenine sites, and methylation-sensitive restriction enzymes to identify altered DNA methylation and epigenetic modifications.\(^{13}\) Direct and serial measures of single carbon pathway enzyme activity in circulating cells, assays of leukocyte performance, parallel assays of cellular genotoxicity, RNA and protein expression, and changes in global and specific DNA, histone and protein methylation before and at intervals after nitrous oxide exposure are well-established laboratory methods that may be performed in the cells of human participants to resolve the mechanisms of toxicity at the molecular level. Finally, leukocyte aliquots should be cryopreserved at each test interval for future genomic, epigenomic, and genotoxic analysis with improved methods, and to address questions yet to be framed.

The present report of genotoxicity adds to mounting evidence against routine anesthetic maintenance with nitrous oxide. Paradoxically, as anesthesia caregivers abandon its everyday use, caregivers in other specialties are introducing or resurrecting nitrous oxide in emergency care, pediatric sedation, “painless” dentistry, and labor and delivery.\(^{14}\) Investigation of comet assay genotoxicity after nitrous oxide use in these settings might be of particular interest in, for example, blood samples from mother and cord after labor analgesia with and without nitrous oxide.

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References


Paul Meyer Wood Learns the Drill

Titled “Section Drill, U.S.A.A.C., Allentown, Pa.,” this postcard (above) depicts the basic training experienced by U.S. Army Ambulance Corpsmen, such as Columbia medical student Paul Meyer Wood. As with most military preparation of recruits, basic training (including lining up and marching in formation) preceded specialized instruction. Fortunately for Wood, his status as a college graduate was rewarded with an Army rank of Lieutenant before his transfer to the Italian Front during World War I. (Copyright © the American Society of Anesthesiologists, Inc.)

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