Reversible nitrous oxide myelopathy and a polymorphism in the gene encoding 5,10-methylenetetrahydrofolate reductase

H. J. Lacassie1,3*, C. Nazar1, B. Yonish4, P. Sandoval2, H. A. Muir3 and P. Mellado2

1Department of Anesthesiology and 2Department of Neurology, Pontificia Universidad Católica de Chile, Santiago, Chile. 3Department of Anesthesiology and 4Department of Obstetrics & Gynecology, Duke University Medical Center, Durham, NC, USA

*Corresponding author: Department of Anesthesiology, Pontificia Universidad Católica de Chile, Marceola 367, Santiago 833-0024, Chile. E-mail: lacassie@med.puc.cl

We present a case of a patient who received nitrous oxide on two occasions within a period of 8 weeks and who subsequently developed a diffuse myelopathy, characterized by upper extremity paresis, lower extremity paraplegia and neurogenic bladder. Laboratory testing revealed hyper-homocysteinaemia and low levels of vitamin B12. Because of this uncommon clinical presentation, we analysed the patient’s DNA, and found a polymorphism in the MTHFR gene that is associated with the thermolabile isoform of the 5,10-methylenetetrahydrofolate reductase enzyme, which explained the myelopathy experienced by the patient after being exposed to nitrous oxide. Soon after initiating supplementary therapy with folic acid and vitamin B12, the neurological symptoms subsided.

Br J Anaesth 2006; 96: 222–5

Keywords: anaesthesia, inhalation; anaesthetics gases, nitrous oxide; complications, myelopathy; enzymes, 5,10-methylenetetrahydrofolate reductase; gene, MTHFR; vitamins, folic acid

Accepted for publication: November 6, 2005

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) (Enzyme commission 1.5.1.20) catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate and carbon donor for the re-methylation of homocysteine to methionine.1 Nitrous oxide (N2O) irreversibly oxidizes the cobalt atom of vitamin B12, thereby inhibiting the activity of the cobalamin-dependent enzyme methionine synthase, which catalyses the latter reaction (Fig. 1).2 Methionine, by way of its activated form, S-adenosylmethionine (SAM), is the principal substrate for methylation in many biochemical reactions, including assembly of the myelin sheath, methyl substitutions in neurotransmitters, and DNA synthesis in rapidly proliferating tissues.2

We present a patient with a polymorphism in the gene coding for MTHFR, who presented with an acute myelopathy after being repeatedly exposed to N2O during successive surgical procedures and recovered almost ad integrum after treatment with vitamin supplementation.

Case report

A 52-yr-old male, ASA I, developed symptomatic cervical intervertebral disc herniations and spinal stenosis at C4, C5 and C6, that produced bilateral distal sensory deficit in the C6 and C7 dermatomal distribution. This was further confirmed with electromyography, a computed tomography scan (CT scan) and magnetic resonance imaging (MRI) which showed C4–C5/C5–C6 intervertebral disc herniations and a 8 mm spinal stenosis at C6 level. The patient underwent surgery with general anaesthesia. The drugs used at induction of anaesthesia were etomidate, fentanyl and atracurium. The patient’s trachea was intubated and anaesthesia was maintained with isoflurane and 50% N2O in oxygen for an uneventful procedure that lasted 200 min. At the conclusion of the operation, the tracheal tube was removed, and the patient was transferred awake to the postoperative care unit, with no neurological deficit.

Two weeks after surgery, the patient complained of paraesthesia of the lower extremities associated with an ataxic gait, which got worse in the ensuing 8 weeks. At that time, the surgical team performed a laminectomy on the same area because of worsening of the symptoms. The presumptive diagnosis was spinal stenosis myelopathy. The anaesthetic technique included the use of thiopental, fentanyl and atracurium at induction, oral tracheal intubation and maintenance with isoflurane and 50% N2O in oxygen. The procedure lasted 105 min. Soon after surgery,
neurological examination showed mild quadraparesis, predominantly involving the lower extremities, associated with neurogenic bladder that required an indwelling bladder catheter.

Three weeks after the second operation, the patient was admitted to our institution because of left leg deep vein thrombosis and symptoms of bladder obstruction. The patient exhibited bilateral progressive paraparesis leading to paraplegia. There was a proprioceptive and vibration sensory deficit at T1 and below, hypo-aesthesia to all sensory modalities below T5, an absent patellar reflex, enhanced Achilles tendon reflex, bilateral Babinski’s sign, and neurogenic bladder. Laboratory examination revealed macrocytic anaemia, hyperhomocysteinaemia: 113 μmol litre⁻¹ (normal: 4.0–14.5 μmol litre⁻¹ in men), low plasma vitamin B₁₂ concentration (<60 pg ml⁻¹, normal: 200–900 pg ml⁻¹) and normal plasma folate concentration (9.5 ng ml⁻¹, normal 5.3–14.4 ng ml⁻¹). Imaging studies showed a normal thoraco-lumbar CT scan and a diffuse and extensive hyperintense cervico-thoracic image on T2 weighted spinal MRI, suggesting a diffuse myelopathy.

Five cycles of methylprednisolone had been given since the initial presentation, for a presumptive diagnosis of an inflammatory demyelinating syndrome. After no obvious improvement, the neurologists began a trial with supplementary therapy with vitamin B₁₂ and folic acid, on the basis that the low plasma vitamin B₁₂ concentrations and the prior exposure to N₂O may have contributed to the injury. The patient had a slow but progressive motor recovery but with little change in the sensory deficit and the neurogenic bladder. The patient was discharged home with vitamin B₁₂ and folic acid supplementation, as well as intermittent catheterization of the bladder and physical therapy.

Six months later, the patient had no motor deficit, normal bladder and sphincter functions; however, he still had proprioceptive deficits in both legs, with minimal ataxia and normal deep tendon and plantar reflexes. By this stage his daily activities were unimpaired. At 5 yr follow-up, the neurological exam was unchanged. His current treatment is a monthly injection of vitamin B₁₂ and daily folic acid supplementation. His most recent laboratory results are: homocysteine: 5.7 μmol litre⁻¹ (normal: 4.0–14.5 μmol litre⁻¹ in men); plasma vitamin B₁₂: 1029 pg ml⁻¹ (normal: 200–900 pg ml⁻¹); haematocrit: 40.6% with normal sized and shaped erythrocytes.

Because of this uncommon clinical presentation, we decided to analyse the patient’s DNA for possible abnormalities in the gene coding for MTHFR.

Preparation and sequence analysis of genomic DNA
After Institutional Review Board approval, genomic DNA was isolated from the patient’s blood using standard extraction techniques. Each of the 11 exons of the MTHFR gene located on chromosome 1p36.3, were amplified from genomic DNA by the polymerase chain reaction (PCR) with the use of pre-designed intronic primers that have
been published elsewhere. The patient’s PCR products were bidirectionally sequenced. Purification of the products was performed using a standard PCR Purification Kit (catalog number 28004, Qiagen Inc., Valencia, CA). We used automated fluorescent DNA fragment analysis (ABI GeneScan™), to generate chromatograms which highlight variations in the MTHFR gene, as previously described. Data analysis was performed using VectorNTI Configtexpress™. The chromatograms were aligned with sequence accession number AY338232.

The patient was found to be homozygous for a 677C→T mutation in exon 4 (genebank reference AY338232 Region: 8747), that results in a substitution of valine for alanine at residue 222, corresponding to the thermolabile form of MTHFR [Online Mendelian Inheritance in Man (OMIM) #236250]. No other polymorphisms were found in the ten other MTHFR exons.

Discussion

The thermolabile form of MTHFR (OMIM #236250), is inherited as an autosomal recessive trait, resulting from a 677C→T mutation in exon 4, leading to a substitution of valine for alanine at residue 222. Homozygosity for the common allele 677C→T causes elevations in plasma homocysteine concentration that are associated with at least a 50% decrement in MTHFR enzyme activity. Our patient is homozygous for the 677C→T mutation, which may predispose him to the development of dysfunction of the enzyme when subjected to a further stress in the system, such as repeated exposure to N₂O.

The 677C→T mutation is common in the general population, with a T allele frequency ranging from 12.6 to 57% depending on the population studied. However, the homozygous TT genotype (two 677C→T alleles) is less prevalent as expected. In the Americas, the highest prevalence is in Mexico (32%), intermediate in Caucasians (9–11%) and very low in African Americans (0–3%). In Europe, the frequency ranges from 4 to 7% (in Dutch and Finnish populations) to intermediate values in France and Hungary (8–10%) to higher values in southern Europe (12–15%) in Spain and northern Italy to 20–26% in southern Italy. Our patient’s ethnic background is Hispanic, with Spanish ancestors, which gives him a moderate risk of having the polymorphism.

Since the biochemical characterization of the thermolabile variant of MTHFR in 1991, and its genetic identification in 1995, much interest has been raised upon its association with different disease processes, such as coronary artery disease; neurological deficits such as neural tube defect and spina bifida; and pre-eclampsia. In a previous report, Selzer and colleagues reported the case of a patient who was heterozygous for the thermolabile isofrom of the enzyme, in addition to two other point mutations in the gene, which limited the enzyme’s activity to about 6% of controls. This alteration, associated with the use of N₂O, resulted in a fatal outcome as a result of respiratory arrest most likely secondary to asymmetric cerebral atrophy and severe demyelination, with astrogliosis and oligodendrogial-cell depletion in the midbrain, medulla and cerebellum. In our case, the patient’s outcome was favourable, despite the homozygosity of the trait, the baseline low concentrations of vitamin B₁₂ and the repeated exposure to N₂O. We hypothesize that the neurological deficit that developed soon after surgery was derived from a further decrease in the enzyme activity attributable to the irreversible oxidation of the cobalt atom of vitamin B₁₂ by way of its interaction with N₂O. Unfortunately we did not perform an enzyme activity assay to confirm a dysfunction of the enzyme, leaving this analysis speculative; however, the successful empiric treatment with vitamin B₁₂ and folic acid served as a diagnostic tool, which was supported by the DNA analysis.

Hyperhomocysteinaemia has been recognized as a risk factor for deep vein thrombosis. Both genetic and environmental (e.g. dietary) factors affect homocysteine levels. The pathophysiology is not fully understood; however, good evidence points towards the fact that hyperhomocysteinaemia leads to a proatherogenic and prothrombotic metabolic milieu. Our patient had deep vein thrombosis that was most likely secondary to long-term immobilization attributable to the neurological deficit, associated with a decrease in methionine synthase function as a result of N₂O exposure, leading to hyperhomocysteinaemia. The thermolabile moiety of MTHFR deficiency is the most common isofrom producing hyperhomocysteinaemia, especially when there are low folate plasma concentrations.

Our patient had normal folate levels; however, patients homozygous for the thermolabile MTHFR mutation could have higher daily folate requirements than normal individuals. This case emphasizes the potential benefits of screening for MTHFR variants. There are no suggestive phenotypic features but the combination of high risk ethnic background, a family history and the possibility of poor diet may suggest further investigation. We do not think that our case is sufficient evidence to propose routine preoperative screening of plasma homocysteine concentrations nor for withholding N₂O as part of a general anaesthetic technique; however, the relative risks and benefits of N₂O should always be considered carefully. There is no information on the use of N₂O during pregnancy and fetal MTHFR activity; therefore, no recommendations can be made at this point.

Treatment of hyperhomocysteinaemia is important because of the clear association with cardiovascular disease. Maintaining a low plasma homocysteine is easily achieved through vitamin supplementation. Patients with elevated homocysteine can be treated effectively with multivitamins containing 400 μg day⁻¹ of folic acid supplemented with an additional 400–1000 μg. After 6–8 weeks of vitamin supplementation, plasma homocysteine measurements should be repeated. Continued elevated homocysteine should be
treated with increased doses of supplemental folic acid up to 5 mg day\(^{-1}\).\(^{14}\) Patients homozygous for the thermolabile MTHFR mutation could have higher daily folate requirements than normal individuals.\(^{14}\)

In summary, we present a case of acute neurological deficit possibly triggered by the exposure to \(\text{N}_2\text{O}\) in a patient with hyperhomocysteinaemia, vitamin B\(_{12}\) deficit and a polymorphism in the MTHFR gene (homozygous for the 677C→T), that was reversible after supplementation with vitamin B\(_{12}\) and folic acid after several weeks of profound neurological impairment.

**Acknowledgement**

Special thanks to Professor Phillip Carl, Associate Professor of Pharmacology, University of North Carolina Medical School Chapel Hill, NC, USA, for his support and valuable comments.

**Conflict of interest**. None declared.

**References**

6. McKusick VA. Homocystinuria due to deficiency of N(5,10)-methylenetetrahydrofolate reductase activity. OMIM, 1986