

Nitrous Oxide Induced Elevation Of Plasma Homocysteine And Methylmalonic Acid Levels And Their Clinical Implications

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Abstract

Clinical research done over the last decade has shown that an elevated plasma level of the amino acid homocysteine is an independent risk factor for atherosclerosis, including coronary artery disease, cerebrovascular disease, peripheral vascular disease and venous thromboembolism (1,2,3,4,5,6). Elevated plasma levels of methyl malonic acid are associated with neurological dysfunction (7,8). This article focuses on the role of nitrous oxide anesthesia in elevated plasma homocysteine and methylmalonic acid levels and their clinical implications.

INTRODUCTION

Clinical research done over the last decade has shown that an elevated plasma level of the amino acid homocysteine is an independent risk factor for atherosclerosis, including coronary artery disease, cerebrovascular disease, peripheral vascular disease and venous thromboembolism (1,2,3,4,5,6). Elevated plasma levels of methyl malonic acid are associated with neurological dysfunction (7,8). This article focuses on the role of nitrous oxide anesthesia in elevated plasma homocysteine and methylmalonic acid levels and their clinical implications.

THE EFFECT OF NITROUS OXIDE ANESTHESIA ON PLASMA HOMOCYSTEINE AND METHYLMALONIC ACID LEVELS

Studies have shown a correlation between nitrous oxide and elevated plasma homocysteine and methylmalonic acid levels. An overview of the molecular species of homocysteine and methylmalonic acid and their metabolism will help us to better understand the role of nitrous oxide in elevated plasma homocysteine and methylmalonic acid levels, which eventually result in postoperative myocardial ischemia and neurological deterioration.

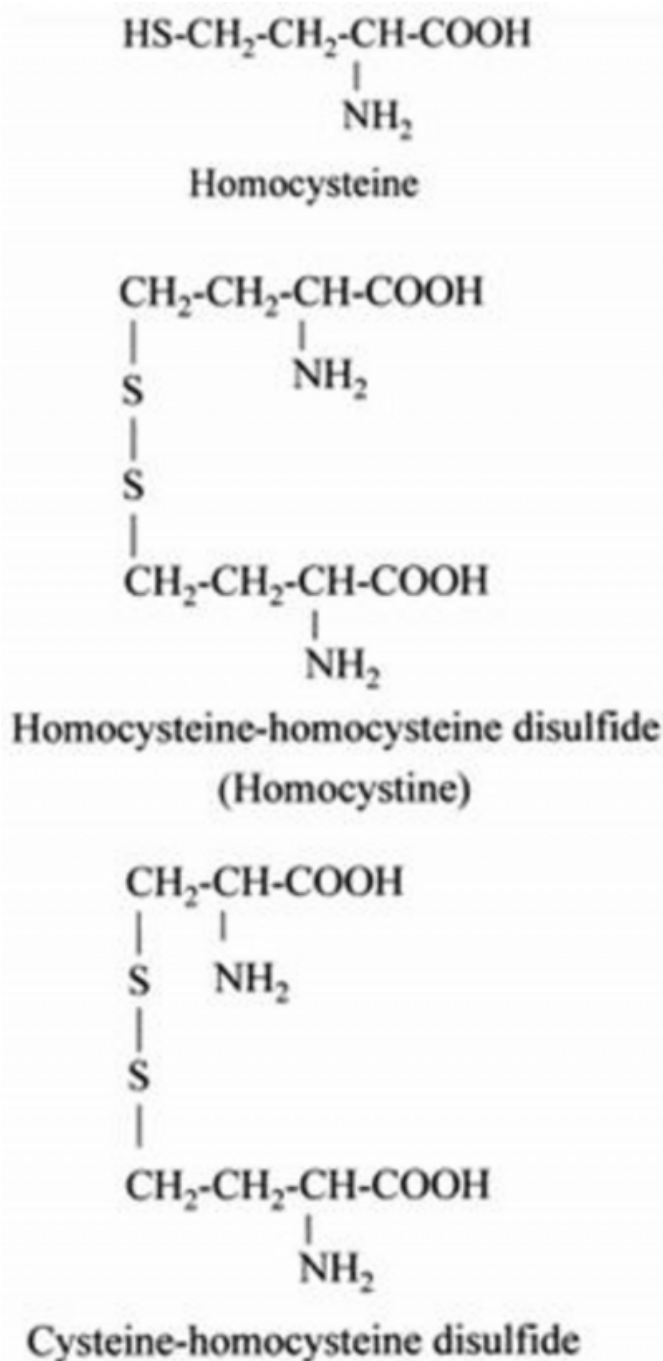
MOLECULAR SPECIES OF HOMOCYSTEINE

Homocysteine is a sulfur-containing amino acid formed during methionine metabolism. Its concentration in the plasma or serum is about 10 $\mu\text{mol/L}$. However, homocysteine exists in various forms; only trace amounts ($\ll 1\%$) are in the

reduced (sulfhydryl) form, the remaining part is oxidized and exists as various disulphides (S_2). It can dimerise to homocystine, or form disulphide bonds with proteins to form so-called 'protein-bound' homocysteine. About 80% of homocysteine is bound to albumin (via a disulphide bond) in the plasma, whereas the remaining 20% exists as free disulphides.

Figure 1

Figure 1: Molecular Species Of Homocysteine.



OUTLINE OF METHIONINE/HOMOCYSTEINE METABOLISM

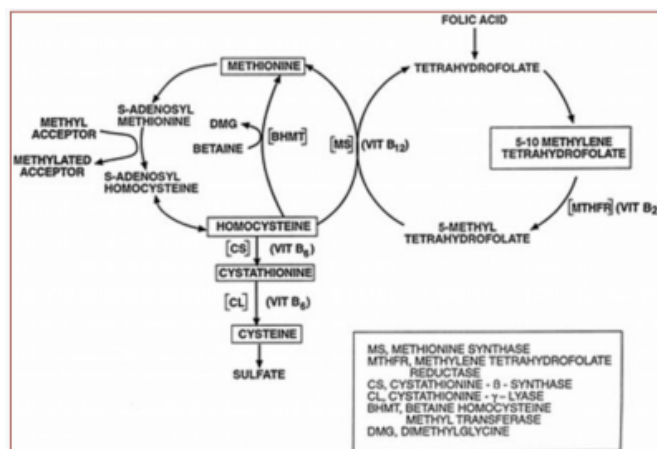
Homocysteine is not a dietary constituent and is not incorporated into proteins but is exclusively formed as an intermediary product of methionine metabolism (₁₀). Through the action of methionine adenosyltransferase, methionine is converted to S-adenosylmethionine (SAM), which is the major biological methyl donor required for

numerous cellular processes, including the formation of proteins, nucleic acids, epinephrine, melatonin, phosphatidylcholine and creatinine (₁₁). These reactions are catalyzed by various methyltransferases that demethylate S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH). S-adenosyl homocysteine (SAH) is hydrolyzed by SAH hydrolase to simultaneously produce homocysteine and adenosine (₁₂).

Once homocysteine is formed, it may be salvaged to methionine by remethylation, or degraded to cysteine by trans-sulfuration. Remethylation to methionine is in most tissues catalyzed by the ubiquitous enzyme, methionine synthase (MS). This enzyme uses vitamin B12 as cofactor, and 5-methyltetrahydrofolate as methyl donor. 5-Methyltetrahydrofolate is formed from folic acid by the vitamin B2-dependent enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR). Reconversion of homocysteine to methionine also contributes to the maintenance of intracellular stores of tetrahydrofolate.

Figure 2

Figure 2: Metabolism Of Methionine/Homocysteine.



Two vitamin B6-dependent enzymes are involved in the trans-sulfuration pathway. The enzyme cystathionine beta synthase (CBS) first condenses homocysteine with serine to form cystathionine, which is then cleaved into cysteine and L-ketobutyrate by cystathionine lyase. Cysteine may be utilized in the synthesis of proteins or as a precursor of the antioxidant glutathione. The trans-sulfuration of homocysteine to cysteine is irreversible, and therefore directs homocysteine to catabolism via cysteine to sulphates as the final product.

Under normal metabolic circumstances, there is a strict balance between homocysteine formation and elimination.

Usually about 50% of the homocysteine formed is remethylated to methionine. When protein or methionine intake is in excess, the trans-sulfuration pathway catabolizes a larger proportion (₁₀). If there is an increased formation of homocysteine relative to its consumption, homocysteine is excreted from the cells. This can be detected as an increased level of homocysteine in plasma/serum or in the urine.

Thus, homocysteine, an intermediate in protein metabolism, is involved in conversion of the amino acid methionine to cysteine or in remethylation to form methionine. Metabolism of homocysteine is by pathways, which re-methylate it (and which require vitamin B12, B2 and folic acid), or by a trans-sulfuration pathway, which requires vitamin B6. Methionine in the diet is the main source of homocysteine in the blood. Animal proteins provide about three times as much methionine as plant protein. Homocysteine in blood (and elsewhere) is a product of how much methionine is eaten, mainly in protein, and how much is metabolized, which in turn may be affected by amounts of B vitamins (B2, B6 and B12) and folate available.

ETIOLOGY OF ELEVATED HOMOCYSTEINE LEVELS

Elevated homocysteine levels are often the result of decreased activity of key enzymes involved in conversion of the amino acid methionine to cysteine (Cystathionine beta synthase and Cystathionine lyase) or in remethylation to form methionine (Methionine synthase and 5,10-methylene tetrahydrofolate reductase (MTHFR)). A plasma homocysteine concentrations exceeding 15 $\mu\text{mol per L}$ is now termed “hyperhomocysteinemia” (₁₃). Normal levels of fasting plasma homocysteine are considered to be between 5 and 15 $\mu\text{mol/L}$. Moderate, intermediate, and severe hyperhomocysteinemia refer to concentrations between 16 and 30, between 31 and 100, and >100 $\mu\text{mol/L}$, respectively (₁₄). The most common inherited form of hyperhomocysteinemia results from an alteration in the gene encoding the enzyme methylene tetrahydrofolate reductase. Less often, the cause of hyperhomocysteinemia is heterozygous cystathionine b-synthase deficiency. Homozygous form of cystathionine b-synthase deficiency results in Homocystinuria, which is a rare but severe condition in which total homocysteine concentrations generally exceed 100 $\mu\text{mol per L}$, sometimes even reaching levels upto 500 $\mu\text{mol per L}$ if the disorder is untreated. Individuals with this inherited disorder are known to have severe hyperhomocysteinemia and a variety of

abnormalities, including a high incidence of vascular pathology that may result in early death from myocardial infarction, stroke, or pulmonary thromboembolism.

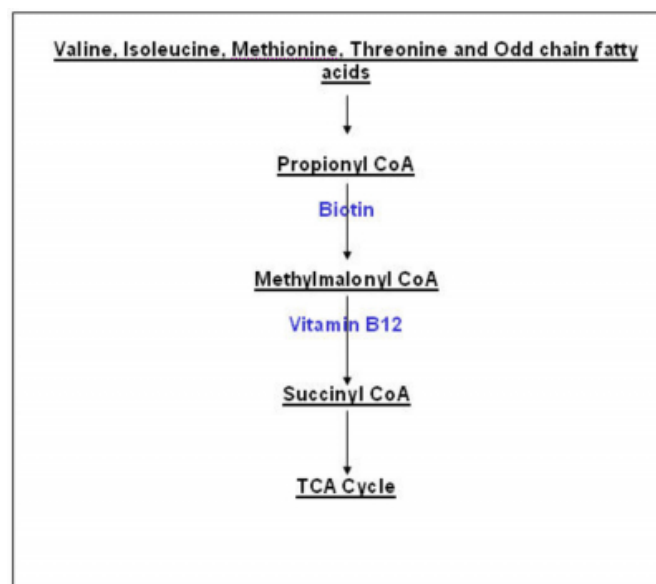
Hyperhomocysteinemia can be acquired as the result of dietary deficiencies of folate, B vitamins (B2, B6 and B12). These vitamins are necessary cofactors for the optimal function of 5,10-methylene tetrahydrofolate reductase, methionine synthase and cystathionine b-synthase. Deficiencies in the absorption or transport of these vitamins can also cause hyperhomocysteinemia.

METHYLMALONYL COA AND ITS METABOLISM

Valine, isoleucine, methionine and threonine are glucogenic amino acids i.e., they can be converted to glucose via tricarboxylic acid (TCA) cycle. These amino acids enter the TCA cycle as succinyl CoA. Methylmalonyl CoA is formed as an intermediate in the conversion of these four glucogenic aminocids to succinyl CoA. The conversion of methylmalonyl CoA to Succinyl CoA is catalyzed by the enzyme methylmalonyl CoA mutase, which requires vitamin B12 as a cofactor. A deficiency of vitamin B12 can result in the accumulation of methylmalonic acid leading to a condition known as methylmalonic aciduria characterized by neurological dysfunction.

Figure 3

Figure 3: Metabolism Of Methylmalonyl CoA.



THE ROLE OF NITROUS OXIDE IN ELEVATED PLASMA HOMOCYSTEINE AND METHYLMALONIC ACID LEVELS

Nitrous oxide can cause hyperhomocysteinemia by irreversibly oxidizing the cobalt atom of vitamin B12

(cofactor for methionine synthase) leading to inhibition of the enzyme methionine synthase, which is involved in the remethylation of homocysteine to methionine. Nitrous oxide induced inactivation of methionine synthase has been demonstrated with purified enzyme (¹⁵), in cultured fibroblasts (¹⁶), human glioma cell lines (¹⁷), rat models (¹⁸) and in humans (^{19,20,21}). The mean half-time of inactivation of methionine synthase by nitrous oxide is 46 minutes. Residual methionine synthase activity approaches zero more than 3 hours after the start of nitrous oxide administration (²⁰). Rats, mice and pigs exposed to nitrous oxide have delayed recovery of methionine synthase enzyme activity for periods of four days or more (^{18,22,23,24}). Nitrous oxide anesthesia induced increase in postoperative homocysteine concentrations is associated with myocardial ischemia (^{25,26}).

Nitrous oxide induced irreversible oxidation of cobalt atom of vitamin B12 also decreases the activity of vitamin B12, leading to impaired activity of methylmalonyl CoA mutase, which is involved in the conversion of methylmalonyl CoA to Succinyl CoA and requires vitamin B12 as a cofactor. This leads to a build up of methylmalonic acid in the blood, which causes neurological deterioration.

An 80-minute period of anesthesia with nitrous oxide has been shown to cause neurological deterioration six days after the anesthetic administration in a child with elevated homocysteine levels due to severe dietary vitamin B12 deficiency (²⁷). This is probably due to increased plasma methylmalonic acid level caused by nitrous oxide induced inhibition of vitamin B12, which is a cofactor for methylmalonyl CoA mutase, involved in the conversion of methylmalonyl CoA to succinyl CoA

Anesthesia with 60 % nitrous oxide on two occasions, a few days apart have been shown to cause neurological deterioration and death in a child with elevated homocysteine levels due to 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency (an inborn error of metabolism) (²⁸). This is probably due to increased plasma methylmalonic acid level caused by nitrous oxide induced inhibition of vitamin B12. An autopsy done on this child showed an asymmetric cerebral atrophy and severe demyelination, with astrogliosis and oligodendroglial-cell depletion in the midbrain, medulla, and cerebellum (²⁸).

CONCLUSIONS

Nitrous oxide constitutes a major component in most of the anesthesia procedures practiced all over the world. Based on

the current information available from the human and animal research studies, we believe that patients with a diagnosis of severe 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency should not receive nitrous oxide as anesthesia. Blood homocysteine assays by High Performance Liquid Chromatography (HPLC) should be considered before using nitrous oxide as anesthesia in patients with a personal or family history of cardiovascular disease, but in whom the well-established risk factors for cardiovascular disease such as smoking, high blood cholesterol, high blood pressure, diabetes, physical inactivity and obesity do not exist. If these patients show elevated homocysteine levels, further work up for the etiology of elevated homocysteine levels should be done before using nitrous oxide as anesthesia. In patients with B vitamin complex (B6, B12 and Folate) deficiency as the cause of elevated homocysteine levels, a one-week course of oral B vitamins can prevent the postoperative increase in homocysteine from nitrous oxide, and, by implication, myocardial ischemia as well (²⁹). Patients with suspected B12 deficiency (megaloblastic anemia and neurological dysfunction) should undergo serum B12 and methylmalonic acid assays before using nitrous oxide as anesthesia to prevent postoperative morbidity and mortality due to myocardial ischemia and neurological deterioration resulting from elevated plasma homocysteine and methylmalonic acid levels respectively. If B12 deficiency is diagnosed, the patient should receive a one-week course of B vitamins before using nitrous oxide to prevent postoperative complications such as myocardial ischemia and neurological dysfunction.

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