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The interaction between nitrous oxide and cobalamin

Biochemical effects and clinical consequences

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Nitrous oxide (N_2O) has been extensively used as an anaesthetic agent since the middle of the nineteenth century. It has been regarded as an ideal drug with few side effects (1). In 1956, Lassen et al. (2) reported that nitrous oxide inhalation for five to six days to control spasms in tetanus patients produced severe bone marrow depression. Twelve years later, Amess and colleagues (3) more directly demonstrated interference with DNA synthesis in bone marrow from patients exposed to nitrous oxide for 24 hours, and they correctly suggested that nitrous oxide may oxidise cobalamin required by methionine synthase (Fig. 1). This mechanism was confirmed by Deacon et al. (4) shortly afterwards. The same year Layzer (5) reported on myeloneuropathy in 15 dentists who had abused nitrous oxide for months to years. Thus, prolonged exposure to nitrous oxide seems to produce haematological and central nervous effects resembling those observed in cobalamin deficiency. This possibility and its biochemical basis have been substantiated by both experimental and clinical studies which are briefly reviewed in this article.

COBALAMIN AS ENZYME CO-FACTOR

Cobalamin serves as cofactor in two known enzymes in humans (Fig. 1). Methylmalonyl-CoA mutase converts methylmalonyl-CoA to succinyl-CoA, and in this enzyme, adenosylcobalamin serves as co-factor (6). The other cobalamin-dependent enzyme is methionine synthase which remethylates homocysteine to methionine. This reaction is coupled to the conversion of 5-methyltetrahydrofolate to tetrahydrofolate, and the prosthetic group turns over between methylcobalamin (which donates the methyl group to homocysteine) and the unstable cob(I)alamin (7).

INACTIVATON OF METHIONINE SYNTHASE BY NITROUS OXIDE

Studies with isolated methionine synthase suggest that nitrous oxide oxidises the highly reactive enzyme-bound cob(I)alamin, leading to product(s) that covalently modify and thereby damage the enzyme (8). The model predicts that enzyme inactivation is dependent on the catalytic turnover (7).

The kinetics of inactivation of methionine synthase by nitrous oxide have been studied in experimental animals and patients *in vivo*. In rodents, the half-life is shorter than 10–30 minutes (9, 10), whereas the rate of inactivation is slower in human subjects, i.e. about 50% inactivation after 1 hour (9, 11). Methionine synthase is the primary target of nitrous oxide, and the cobalamin-dependent methylmalonyl-CoA mutase is not directly affected. Notably, the inactivation is irreversible, and recovery of enzyme activity is probably dependent on synthesis of new enzyme (8) which requires 3-4 days in humans (9).

Results from experiments with cultured cells treated with nitrous oxide showed that high content of folate in the medium enhances the methionine synthase inactivation (12), whereas high medium methionine protects the enzyme (13). These findings agree with the model predicting that high catalytic activity of the enzyme favours the inactivation process (7), and may infer strategies to promote (simultaneous folate administration) or protect (methionine administration) against enzyme inactivation.

METABOLIC EFFECTS AND BIOCHEMICAL MARKERS

Serum cobalamin remains stable in patients after exposure to nitrous oxide for 24 hours (3), and is not a

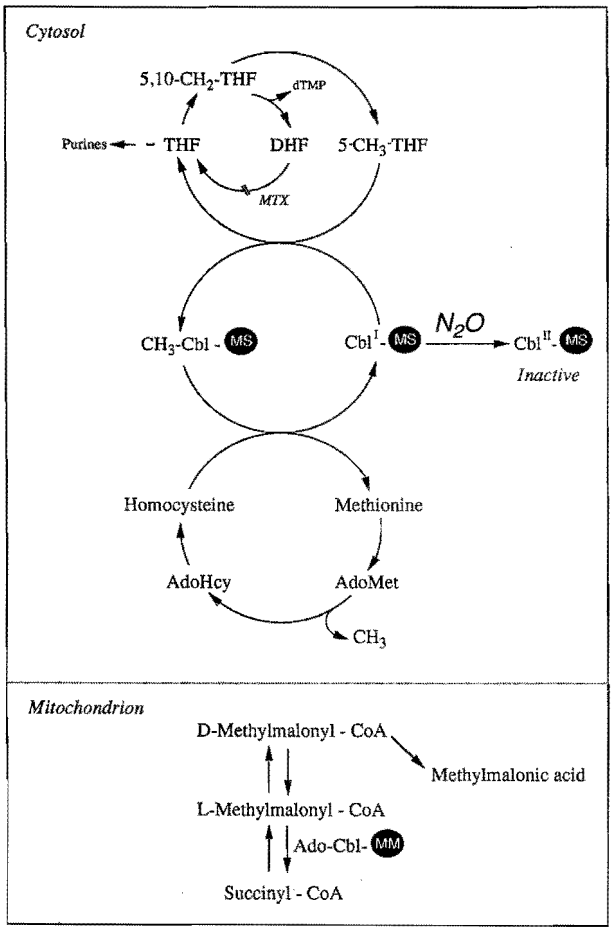

Fig. 1. Cobalamin-dependent reactions and the interference by nitrous oxide. Nitrous oxide oxidizes cobalamin bound to methionine synthase, but does not directly affect the other cobalamin dependent reaction. AdoHcy=adenosylhomocysteine, AdoMet=adenosylmethionine, Cbl=cobalamin, Cbl^{I}=monovalent cobalamin, Cbl^{II}=divalent cobalamin, CH_3=methyl, DHF=dihydrofolate, MM=methylmalonyl-CoA mutase, MS=methionine synthase, THF=tetrahydrofolate.

useful marker of impaired cobalamin function under this condition. Some decrease in serum cobalamin after prolonged nitrous oxide anaesthesia might be expected, since cobalamin analogues are formed. These are preferentially excreted (14), and they are not detected by most cobalamin assays used today.

Alterations in the tissue level and thereby the plasma concentrations of the substrates (homocysteine and 5-methyltetrahydrofolate) and product (methionine) of the methionine synthase reaction are conceivable results of nitrous oxide exposure. Plasma homocysteine is significantly raised already after 75 minutes of nitrous oxide anaesthesia, and exposure for 5 hours, 24 hours and 4 days has been shown to increase plasma homocysteine 80%, 2–3 fold and 10–15 fold, respectively. The maximum level is reached after days and usually remains elevated for at least one week (15, 16). A dentist, who had abused nitrous oxide for years, had a 10-fold elevation of plasma homocysteine (16). Thus, plasma homocysteine is a responsive and sensitive indicator of cobalamin oxidation. Variability in homocysteine response between individuals is observed, which is partly related to different folate and possibly cobalamin status before anaesthesia (15).

The hyperhomocysteinaemia is associated with a concurrent decrease in plasma methionine (9), and a shorter, transient increase in serum folate (15, 16). Changes in serum folate reflect decrease in the intracellular content of tetrahydrofolates, which has been demonstrated both in experimental animals and in patients (17). This is explained by trapping of intracellular folate as 5-methyltetrahydrofolate at the expense of the other reduced folate species (9). Impaired folate homeostasis explains increased urinary excretion of formiminoglutamic acid (18), and inhibition of thymidylate and thereby DNA synthesis, as demonstrated by the deoxyuridine suppression test (9).

Methylmalonyl-CoA mutase is decreased after prolonged (33 days) nitrous oxide exposure in rats, probably due to formation of cobalamin analogues and redistribution of cobalamin species (14). This remote metabolic effect of nitrous oxide explains elevated serum methylmalonic acid after long-term abuse of nitrous oxide (16).

EFFECTS ON THE HAEMATOPOIETIC AND CENTRAL NERVOUS SYSTEM

There are consistent reports of impaired haematopoiesis in patients exposed to nitrous oxide. These changes range from subtle biochemical effects and megaloblastic bone marrow to severe marrow depression, anaemia and death. The severity of the marrow depression is dependent on both the duration of exposure and the concentration of nitrous oxide, and cobalamin status of the afflicted patient (9).

Carmel et al. (19) demonstrated mild impairment of DNA synthesis in bone marrow cells harvested for transplantation from donors given nitrous oxide for only 75–120 minutes. Bone marrow dysfunction as assessed by deoxyuridine suppression test and bone marrow morphology usually develops after more than 4–6 hours of nitrous oxide exposure of the uncompromised patient, but is occasionally absent after 24 hours of exposure. The changes usually resolve within 1–10 days (9).

In patients without cobalamin deficiency, severe depression of bone marrow function can be expected after prolonged (>24 hours) nitrous oxide anaesthesia. Exposure for 24–36 h results in neutropenia within 2–4 days followed by increased proportion of hyperseg-

mented polymorphs in blood. Exposure for 4 days or longer usually results in agranulocytosis (9).

Studies in both animals and man have shown that the effect of nitrous oxide on methionine synthase is enhanced by cobalamin deficiency, and cobalamin status influences the inactivation of the enzyme *in vivo* (20). Thus, the cobalamin-deficient patient may be particularly susceptible to the adverse effects of nitrous oxide. There are also indications that debilitated patients develop bone marrow changes faster than healthy subjects (9).

Notably, myelopathy has been reported in five patients with unsuspected cobalamin deficiency following nitrous oxide anaesthesia of less than 4 hours duration (21). Megaloblastic bone marrow changes have been observed after less than 2 hours of nitrous oxide exposure in critically ill patients (22).

Clinical outcome of chronic intermittent use of nitrous oxide is less damaging than might be expected, particularly in children (9), and there seems to be a homeostatic mechanism protecting the bone marrow against intermittent exposures (23). There are several reports on severe myeloneuropathy in health personnel abusing nitrous oxide (5, 24). Notably, only two of 32 reported abusers of nitrous oxide with myeloneuropathy had some haematological involvement (24).

PRECAUTIONS AND TREATMENT

Nitrous oxide exposure should be restricted to less than 24 hours. In debilitated or cobalamin-deficient patients, nitrous oxide exposure for 2–6 hours may lead to megaloblastic bone marrow changes (22), and nitrous oxide should either be avoided or proper measures carried out. Supplement of cobalamin, folinic acid and/or methionine administration have been recommended both as treatment and prevention of nitrous oxide induced cobalamin deficiency (9, 21, 24).

In patients who have developed clinical symptoms after nitrous oxide exposure, cobalamin supplementation can improve the haematological disturbances (21, 24, 25), whereas myeloneuropathy is reversed in some (21), but not all cases (24). Administration of cobalamin in close association with nitrous oxide exposure may lead to increased formation of cobalamin analogues, which are excreted in the urine, and may function as cobalamin antagonists (14).

Folinic acid (30 mg twice daily) seems to prevent the development of abnormal deoxyuridine suppression test and megaloblastic marrow (9), probably by circumventing the methyltetrahydrofolate trap. The timing of folinic acid supplementation may be critical since *in vitro* data suggest that excess folate makes methionine synthase more susceptible to inactivation, possibly by increasing the catalytic turnover of the enzyme (12). These observations may indicate that folinic acid administration after termination of anaesthesia is preferable.

Methionine administration protects experimental animals against neurological impairments caused by nitrous oxide (26, 27), and may enhance the recovery from myeloneuropathy in humans (24). The beneficial effects of methionine may be related to protection (13) or restoration of methionine synthase activity (28), or to provision of methyl groups for the methylation of myelin basic protein (29).

The haematological changes induced by nitrous oxide are probably due to impaired folate dependent thymidylate and purine synthesis (9), and patients with preexisting folate deficiency may be more susceptible to the effects of nitrous oxide. This is supported by observations that breast cancer patients receiving the antifolate drug methotrexate in association with nitrous oxide anaesthesia sometimes develop an unexpected myelosuppression (30). *In vitro* studies suggest that nitrous oxide aggravates the effect of methotrexate on intracellular folate metabolism (31). The combination of nitrous oxide and methotrexate should therefore be avoided (32). Low serum folate before anaesthesia is associated with increased homocysteine response to nitrous oxide (15), but a relation between folate deficiency and haematological or neurological disturbances induced by nitrous oxide has not been reported.

The present state of knowledge does not justify firm recommendations on how to identify patients at risk, how to prepare them for nitrous oxide anaesthesia, and the best intervention to prevent complications. Determination of vitamin levels, methylmalonic acid and/or homocysteine in serum or plasma can reveal a deficiency or disturbed function of cobalamin or folate. Notably, recent studies have shown that deficiencies of these vitamins are more common than previously recognised, and, among the elderly, as many as 30-40% have an elevated level of plasma homocysteine and/or serum methylmalonic acid (33). Further studies are required to investigate whether these patients may be at increased risk of developing bone marrow changes during nitrous oxide anaesthesia.

CONCLUSION

In patients with normal preoperative cobalamin and folate function, the effect of short term nitrous oxide exposure on cobalamin metabolism causes no clinical sequelae, and the interaction should not undermine its position as a safe analgesic and anaesthetic agent.

Debilitated, cobalamin-deficient and possibly folate-deficient patients are more susceptible to the adverse effects of nitrous oxide, and prophylactic supplement of

cobalamin and/or folinic acid seems justified. Determination of plasma homocysteine and serum methylmalonic acid may represent convenient laboratory tests for pre- and postoperative evaluation of these patients.

The recent data that biochemical deficiency of cobalamin and/or folate is fairly common, especially among the elderly, will hopefully incite new clinical investigations on patients undergoing nitrous oxide anaesthesia, and can possibly lead to more comprehensive recommendations regarding identification and treatment of persons at risk.

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