PREVENTION OF NITROUS OXIDE-INDUCED MEGALOBLASTIC CHANGES IN BONE MARROW USING FOLINIC ACID

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SUMMARY

Prolonged anaesthesia with nitrous oxide inactivates vitamin B₁₂ and impairs DNA synthesis in bone marrow cells. The use of parenteral folinic acid in the prevention of these toxic effects has been studied in 11 patients, ventilated artificially with nitrous oxide in oxygen for 24 h. Bone marrow aspirates were performed before and after exposure to nitrous oxide. They were assessed morphologically and with the deoxyuridine suppression test. Folinic acid 30 mg immediately before anaesthesia and 30 mg 12 h later, prevented the toxic effects of nitrous oxide in four out of five patients, whereas smaller amounts of folinic acid (between 3 and 36 mg in 24 h), were ineffective.

Lassen and colleagues (1956) reported that, in patients with tetanus, prolonged anaesthesia with nitrous oxide produced severe bone marrow depression with megaloblastic changes in the bone marrow which were similar to those seen in untreated pernicious anaemia. Leucopenia and thrombocytopenia contributed to the deaths of two of their patients. These observations were confirmed by Amess and co-workers (1978), who demonstrated impaired DNA synthesis in the bone marrow cells of patients undergoing cardiopulmonary bypass surgery, and artificial ventilation with nitrous oxide for a total of 24 h. The aspirates of bone marrow were all megaloblastic and the deoxyuridine (dU) suppression tests indicated that this was as a result of inactivation of vitamin B₁₂. In the same study, patients anaesthetized with nitrous oxide for shorter periods of time, 5–12 h, had less consistent morphological abnormalities and less marked changes in the dU-suppression test.

The majority of routine surgical procedures, in which nitrous oxide is used, are considerably shorter than this, and are unlikely to produce significant changes in the bone marrow (Kano et al., 1981; O'Sullivan et al., 1981). However, in critically ill patients admitted to an intensive care unit (ICU) following anaesthesia with nitrous oxide for 1–6 h, Amos and colleagues (1982) noted relatively severe changes in the bone marrow, recovery from which was prolonged considerably when compared with patients undergoing cardiopulmonary bypass surgery. Among the patients receiving intensive care, abnormalities in the dU-suppression test were present following as little as 1 h anaesthesia with nitrous oxide and, in the most seriously ill patients, megaloblastic bone marrow change was present after anaesthesia of only 2–4 h duration.

Nitrous oxide is a useful anaesthetic agent in seriously ill patients and it would be valuable to have a means of preventing the bone marrow toxicity associated with its use. In vitro the abnormal dU-suppression test produced by exposure to nitrous oxide is partially corrected by the addition of cyanocobalamin or pteroylglutamic acid (PGA) and fully corrected by folinic acid (5-formyltetrahydrofolate) (Deacon et al., 1980a). O'Sullivan and colleagues (1981) successfully used folinic acid to prevent the development of morphological abnormalities and changes in the dU-suppression test in seven patients ventilated with nitrous oxide for 24 h. They gave folinic acid 20 mg by mouth 12 h, and 1 h, before the induction of anaesthesia. To investigate the possibility of using folinic acid to prevent these nitrous oxide-induced changes in bone marrow in emergency surgical patients and patients in the ICU, folinic acid was administered parenterally, in varying doses, immediately before the induction of anaesthesia and again 12 h later. The study was approved by the Hospital Ethics Committee, and informed consent was obtained either from the patient or a close relative.

PATIENTS AND METHODS

Eleven patients were ventilated artificially with 50%...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Folinic acid</th>
<th>Before nitrous oxide</th>
<th>Following nitrous oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total dose (mg)</td>
<td>Bone marrow morphol.</td>
<td>% dU-suppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*Time (h) Dose (mg) +B12 +PGA</td>
<td>dU</td>
<td>+B12</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>M</td>
<td>MVR:AVR</td>
<td>0 3 3</td>
<td>NB 2.7 2.6 2.3</td>
<td>MB 22.9 20.2 11.5 5.5</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>M</td>
<td>CAVBG</td>
<td>0 9 9</td>
<td>NB 3.1 2.7 2.6</td>
<td>MB 18.2 9.2 6.5 —</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>M</td>
<td>CAVBG</td>
<td>0 9 18</td>
<td>NB 1.6 1.4 1.3</td>
<td>MB 21.6 10.7 7.7 —</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>F</td>
<td>AVR</td>
<td>0 12 24</td>
<td>NB 4.2 3.9 3.8</td>
<td>MB 19.9 11.2 9.0 —</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>Peptic ulceration</td>
<td>0 12 12</td>
<td>NB 4.6 4.6 4.3</td>
<td>MB 25.1 14.8 9.0 —</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>M</td>
<td>CAVBG</td>
<td>0 6 9 9 36</td>
<td>NB 3.3 3.1 3.0</td>
<td>MB 24.7 18.2 20.6 8.5</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>CAVBG</td>
<td>0 12 30 30</td>
<td>NB 2.1 1.6 2.3</td>
<td>NB 6.7 5.2 4.3 —</td>
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<tr>
<td>8</td>
<td>54</td>
<td>F</td>
<td>MVR</td>
<td>0 12 30 30</td>
<td>NB 4.0 3.7 3.6</td>
<td>NB 6.0 5.0 3.7 —</td>
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<tr>
<td>9</td>
<td>49</td>
<td>M</td>
<td>Meningitis</td>
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<td>10</td>
<td>54</td>
<td>M</td>
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<td>0 12 30 30</td>
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<td>11</td>
<td>52</td>
<td>M</td>
<td>Liver abscess</td>
<td>0 12 30 30</td>
<td>NB 5.1 5.1 4.6</td>
<td>MB 15.8 8.2 4.0 —</td>
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nitrous oxide in oxygen for 24 h. Seven patients underwent cardiopulmonary bypass surgery, during which they were anaesthetized with nitrous oxide, and following which ventilation with nitrous oxide was continued for a total of 24 h. In the remaining four patients nitrous oxide was administered to facilitate medical and nursing management on the ICU. Details of the patients are shown in table I.

Each patient was treated with parenteral folinic acid. The total dose of folinic acid varied from 3 to 60 mg in 24 h. Patients received folinic acid immediately before the induction of anaesthesia, and at varying times during anaesthesia (table I).

A full blood count, including examination of a peripheral blood film, estimation of serum vitamin B₁₂ concentration, serum and red cell folate concentrations and a bone marrow aspirate were taken immediately before the beginning of nitrous oxide anaesthesia, and repeated 24 h later, at the completion of the period of anaesthesia. Full blood counts were performed on a Coulter S-plus counter. Serum vitamin B₁₂ concentration (Anderson, 1964), and serum and red cell folate concentrations (Millbank et al., 1970) were determined by microbiological assay. The bone marrow aspirate was assessed morphologically and a dU-suppression test performed. The method used for the dU-suppression test was that of Wickramasinghe and Longland (1974) with minor modifications (Amess et al., 1978). This test involved the short-term culture in vitro of bone marrow cells. The uptake of tritiated thymidine by bone marrow cells in the control tubes, which lack deoxyuridine, represents 100% uptake of the labelled material. In normal bone marrow the uptake of tritiated thymidine is suppressed to less than 5% of the control tube values by the addition of deoxyuridine. In vitamin B₁₂ or folate deficiency, or under conditions which interfere with the activity of these vitamins, the uptake of deoxyuridine is defective and a relatively large amount of tritiated thymidine is taken up and incorporated into DNA. In megaloblastic anaemia resulting from vitamin B₁₂ deficiency, the incorporation of deoxyuridine into DNA is partially corrected by the addition of either cyanocobalamin or PGA in vitro, whereas in folate deficiency only PGA produces partial correction (Metz et al., 1968). Folinic acid has been shown to be more effective than either cyanocobalamin or PGA in correcting the abnormal dU-suppression test in patients with vitamin B₁₂ deficiency (Deacon et al., 1980b).

### RESULTS

#### Before administration of nitrous oxide

Three patients were anaemic before nitrous oxide anaesthesia; however, the mean red cell volume (MCV) was normal in all patients and macrocytic red cells were not present on the peripheral blood films. The neutrophil counts and platelet counts were normal in all patients.

No patient had subnormal serum vitamin B₁₂, or serum or red cell folate, concentrations, before nitrous oxide anaesthesia, although in two patients the serum folate assay was inhibited by antibiotic chemotherapy.

The bone marrow aspirates were all normoblastic and the dU-suppression tests were normal in all patients except number 10, who had a dU-suppression value of 13.3% (table I). This patient had been anaesthetized with nitrous oxide for 2.5 h the previous day, and the dU-suppression test was compatible with previous findings in ICU patients anaesthetized with nitrous oxide for 2—4 h.

#### Following administration of nitrous oxide

After ventilation with nitrous oxide for 24 h no patient had an increased MCV, and no patient was neutropenic. Five of the seven patients who had undergone cardiopulmonary bypass surgery were thrombocytopenic. However, the period of thrombocytopenia was not prolonged and the platelet count was normal in all patients 5 days later.

No patient had a subnormal serum vitamin B₁₂ concentration, or red cell folate concentration following nitrous oxide anaesthesia. Treatment with folinic acid had increased the serum folate concentration to greater than the normal range in all patients except two, in whom the assay was inhibited by antibiotic chemotherapy.

Patients 1—6 received a total dose of 3—36 mg of folinic acid, given parenterally, in a variety of dose schedules. In each patient the bone marrow aspirate after nitrous oxide was megaloblastic with an abnormal dU-suppression test (mean = 22.1%; SEM ± 3.0%), which was partially corrected in vitro by the addition of cyanocobalamin, PGA or folinic acid (table I). The addition of folinic acid in vitro to the dU-suppression test was more effective than either cyanocobalamin or PGA in correcting the abnormality produced by nitrous oxide.

Patients 7—11 were each given parenteral folinic acid 60 mg in divided doses, at the beginning of and 12 h after the start of anaesthesia. In four patients this prevented the development of nitrous oxide-
induced bone marrow toxicity, all four had a normoblastic bone marrow and only minimally abnormal dU-suppression tests (mean 6.9%; SEM ± 0.8%) (table I). The exception was patient 11 who, despite receiving folinic acid 60 mg, had a megaloblastic bone marrow and an abnormal dU-suppression test of 15.8%, which was corrected in vitro by the addition of cyanocobalamin or PGA.

**DISCUSSION**

These results confirm that prolonged ventilation with nitrous oxide impairs DNA synthesis in human bone marrow cells, resulting in the development of megaloblastic change and an abnormal dU-suppression test. The correction pattern of the abnormal dU-suppression tests indicates that this was a result of the inactivation of vitamin B₁₂. The development of these abnormalities was not prevented by treating patients with folinic acid 3–36 mg in a variety of dose schedules, although increasing the amount of folinic acid to 60 mg did prevent the development of bone marrow abnormalities in four out of five patients. Interestingly, the bone marrow changes were not prevented in one of the seriously ill patients in the ICU. It has been shown previously that these patients develop more severe bone marrow abnormalities than patients undergoing cardiopulmonary bypass surgery, after a similar period of anaesthesia with nitrous oxide (Amos et al., 1982).

Two other groups have used folinic acid to prevent the development of bone marrow toxicity following prolonged exposure to nitrous oxide. O'Sullivan and colleagues (1981) gave folinic acid 40 mg orally in divided doses 12 h and 1 h before the induction of anaesthesia. All of their patients had a normoblastic bone marrow and a normal dU-suppression test after 24 h anaesthesia with nitrous oxide. Skacel and co-workers (1982) were unable to confirm these findings in three patients, all of whom developed abnormalities in the dU-suppression test, despite one patient being treated with folinic acid in the same way as described by O'Sullivan and colleagues (1981). However, in a fourth patient, given folinic acid 30 mg i.v. every 5 h during a 24-h period of exposure to nitrous oxide, the bone marrow remained normoblastic with a normal dU-suppression test.

Folinic acid is a fully reduced form of folate and in vitro corrects the abnormal dU-suppression test resulting from vitamin B₁₂ deficiency (Deacon et al., 1980b) or following vitamin B₁₂ inactivation by nitrous oxide (Deacon et al., 1980a). However, in vivo, folinic acid is only effective when given repeatedly in large amounts. It is possible that nitrous oxide may directly inactivate folinic acid, although we have been unable to demonstrate this in vitro (unpublished observations). Alternatively, nitrous oxide may prevent the uptake of folinic acid into, or its retention within, cells. The hepatic uptake of radioactively-labelled folate analogues was decreased in rats maintained for 24 h in an atmosphere of 50% nitrous oxide (Perry et al., 1979), although this was not confirmed using isolated rat hepatocytes (Horne and Briggs, 1980). However, following hepatic uptake the synthesis of folate polyglutamates, which are the active forms of intracellular folate, from formyl-folates, was normal in the nitrous oxide-treated rat (Perry et al., 1979). If exposure to nitrous oxide does impair cellular uptake of folinic acid, treating patients before anaesthesia should be an effective way of preventing nitrous oxide-induced bone marrow toxicity. This was the experience of O'Sullivan and colleagues (1981), although Skacel and co-workers (1982) could not confirm this.

Prolonged use of nitrous oxide leads to both neutropenia and thrombocytopenia (Lassen et al., 1956). However, ventilation with nitrous oxide for periods of up to 24 h has not been shown to produce these effects (Amess et al., 1978; Skacel et al., 1983). Large doses of folinic acid given immediately before anaesthesia can prevent the development of bone marrow toxicity caused by nitrous oxide, and may be indicated in the management of emergency surgical or ICU patients. However, this is not invariably the case and, in one seriously ill patient ventilated with nitrous oxide, DNA synthesis remained severely impaired, despite treatment with folinic acid 60 mg.

**ACKNOWLEDGEMENTS**

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NITROUS OXIDE-INDUCED BONE MARROW CHANGES

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PREVENTION PAR L'ACIDE FOLINIQUE DES MODIFICATIONS MEGALOBLASTIQUES MEDULLAIRES INDUITES PAR LE PROTOXYDE D'AZOTE

RESUME
Une anesthésie prolongée au protoxyde d'azote inactive la vitamine B12 et altère la synthèse de l'ADN dans les cellules de la moelle osseuse. L'utilisation de l'acide folinique, par voie parentérale dans la prévention de ces effets toxiques, a été étudiée chez 11 patients ventilés artificiellement avec du protoxyde d'azote dans l'oxygène pendant 24 h. Des ponctions médullaires ont été faites avant et après exposition au protoxyde d'azote. Elles ont été à l'origine d'études morphologiques et de tests de suppression de la deoxyuridine. L'administration d'acide folinique 30 mg juste avant l'anesthésie et 30 mg 12 h après, a permis de prévenir les effets toxiques du protoxyde d'azote chez quatre patients sur cinq, alors que des posologies plus faibles (3 et 36 mg par 24 h) n'étaient pas efficaces.

DIE PRÄVENTION LACHGAS-INDUZIERTER MEGALOBLASTEN-VERÄNDERUNGEN IM KNOCHENMARK DURCH FOLSÄURE

ZUSAMMENFASSUNG

PREVENCION DE LOS CAMBIOS MEGALOBLASTICOS INDUCIDOS POR EL OXIDO NITROSO EN LA MEDULA OSEA MEDIANTE EL USO DEL ACIDO FOLINICO

SUMARIO
La anestesia prolongada con óxido nitroso hace inactiva la vitamina B12 y daña la síntesis DNA en las células de la médula ósea. Se estudió el uso del ácido folínico parenteral en la prevención de dichos efectos tóxicos en 11 pacientes, artificialmente ventilados con óxido nitroso en oxígeno durante 24 h. Los productos aspirados de médula ósea fueron tomados antes y después de la exposición al óxido nitroso. Fueron evaluados desde el punto de vista morfológico y mediante el ensayo de supresión de deoxiuridina. Con 30 mg de ácido folínico inmediatamente antes de la anestesia y 30 mg 12 h después se previno los efectos tóxicos del óxido nitroso en cuatro de cinco pacientes, mientras que en cantidades menores, el ácido folínico (entre 3 y 36 mg en 24 h) fue ineficaz.