The effect of sepsis and short-term exposure to nitrous oxide on the bone marrow and the metabolism of vitamin B\textsubscript{12} and folate

S. M. VAN ACHTERBERGH, B. J. VORSTER, A. DU P. HEYNS

Summary

It is recognised that prolonged anaesthesia with nitrous oxide (N\textsubscript{2}O) induces megaloblastic anaemia by oxidising vitamin B\textsubscript{12}. To determine whether sepsis aggravates the effect of N\textsubscript{2}O on haemopoiesis 5 patients with severe sepsis, who required surgery and were exposed to short-term (45 - 105 minutes) N\textsubscript{2}O anaesthesia, were studied. None had evidence of pre-operative vitamin B\textsubscript{12}, or folate deficiency. The effect of the combination of N\textsubscript{2}O anaesthesia and sepsis on DNA synthesis in bone marrow cells was assessed morphologically, and by the deoxyuridine suppression test. In 3 patients exposed to the longest duration (75 - 105 minutes) of N\textsubscript{2}O, addition of folic acid and vitamin B\textsubscript{12} partially improved the utilisation of deoxyuridine in vitro. No patient had evidence of megaloblastic haemopoiesis as judged by bone marrow morphology. It is concluded that prolonged N\textsubscript{2}O anaesthesia in patients with severe sepsis may adversely affect DNA synthesis. Although this effect did not manifest as overt megaloblastic erythropoiesis, it may be prudent to avoid N\textsubscript{2}O in such patients.

A study was undertaken to test the hypothesis that sepsis may contribute to the effect of N\textsubscript{2}O on vitamin B\textsubscript{12} and folate metabolism thus exacerbating the degree of megaloblastic haemopoiesis in those patients with sepsis requiring surgery.

Patients and methods

Five patients with sepsis and requiring surgery were studied in a project approved by the Ethical Committee of the University of the Orange Free State. Patient details are given in Table I. Four patients had abdominal surgery. The exposure to N\textsubscript{2}O varied from 45 minutes to 105 minutes.

The severity of sepsis was graded according to a modification of the classification of Skau et al.\textsuperscript{3} and Lebute and Stoner.\textsuperscript{6} The simple numerical score was based on: type of sepsis, pyrexia, secondary effects of sepsis, and relevant laboratory data (Table I). Only patients with a score of 7 or more were admitted to the study.

Patients were excluded from the study if they: (i) suffered from any haematological disease except anaemia due to haemorrhage; (ii) were treated with drugs known to affect vitamin B\textsubscript{12} or folate metabolism (e.g. anti-epileptic drugs, immunosuppressive medication or chemotherapeutics); or (iii) had clinical or laboratory evidence of chronic liver disease.

Protocol and anaesthesia

The patient's general condition was noted and in preparation for emergency surgery, 10 mg metoclopramide was given intravenously and 15 ml of magnesium trisilicate administered orally. Pre-operative and laboratory tests included measurement of blood gases; a full blood count; assay of red cell and serum folate, and serum vitamin B\textsubscript{12} levels (SimulTRAC-SNB, Becton Dickinson, Orangeburg, NY, USA); a biochemical profile (SMAC, Technicon Instruments); and a routine coagulation screening consisting of measurement of prothrombin and activated partial thromboplastin time.

After pre-oxygenation, sleep was induced with thiopentone 3 mg/kg, cricoid pressure applied, and intubation facilitated with suxamethonium 1,5 mg/kg. Anaesthesia was maintained with low doses of halothane and/or fentanyl. After the aspiration of a bone marrow sample, 50 - 70% N\textsubscript{2}O was included in the anaesthetic.

After surgery, the patients were admitted to an intensive care unit. A bone marrow aspiration was repeated 24 hours later and serum and red cell folate and serum vitamin B\textsubscript{12} levels again estimated. The full blood count and biochemical profile were repeated daily for 7 - 8 days postoperatively.

The DUST was performed on both intra- and postoperative bone marrow aspirates, as described.\textsuperscript{7}

Results

Pre-operatively, 3 patients had a moderate normochromic normocytic anaemia. Patient 2 had a pancytopenia and patients 2 and 4 had a generalised bleeding tendency manifesting as
TABLE I. PATIENT DETAILS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex, age (yrs)</th>
<th>Diagnosis</th>
<th>Sepsis score</th>
<th>Operation</th>
<th>Duration of exposure to N₂O (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 19</td>
<td>Perforated bowel</td>
<td>7</td>
<td>Laparotomy</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>F, 23</td>
<td>Puerperal sepsis</td>
<td>14</td>
<td>Hysterectomy; bilateral salpingo-oophorectomy</td>
<td>75; 45 (2nd laparotomy)</td>
</tr>
<tr>
<td>3</td>
<td>M, 18</td>
<td>Ruptured appendix + abscess</td>
<td>10</td>
<td>Laparotomy; drainage of abscess</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>F, 28</td>
<td>Puerperal sepsis</td>
<td>10</td>
<td>Hysterectomy; bilateral salpingo-oophorectomy</td>
<td>105</td>
</tr>
<tr>
<td>5</td>
<td>M, 23</td>
<td>Bilateral empyema and mediastinitis</td>
<td>10</td>
<td>Drainage of empyema</td>
<td>45</td>
</tr>
</tbody>
</table>

TABLE II. SEPSIS SCORE

2 points
- Chill or fever > 38.9°C or hypothermia < 35.6°C
- Tachypnoea > 28/min or partial arterial carbon dioxide pressure (PaCO₂) < 32 mmHg
- PaO₂ < 60 mmHg (not due to pre-existing lung disease)
- Hypotension < 90 mmHg or tachycardia > 110/min
- Generalised peritonitis or deep-seated infection, e.g. pelvic abscess
- Jaundice (not due to pre-existing liver disease)

1 point
- Metabolic acidosis
- Elevated liver enzyme values
- Oliguria or elevated serum urea or creatinine values
- Thrombocytopenia or evidence of disseminated intravascular coagulation
- Positive blood culture

bleeding at sites of surgical incision and venepuncture. This was ascribed to thrombocytopenia on day 1 and day 2 postoperatively (Table III).

Two patients had a neutrophil leucocytosis and 1 was neutropenic. There was no hypersegmentation of the neutrophil nucleus or shift to the left observed in any of the patients.

All patients had normal vitamin B₁₂ and folate status. This was reflected by normal serum vitamin B₁₂ and red cell folate levels. In patient 3 red cell folate could not be assayed because the blood specimen was lost; however, his serum folate was normal (Table IV).

The dUST was performed on bone marrow samples collected pre-operatively and 24 hours postoperatively. Patient 2 had a second exposure to N₂O and the dUST was repeated 24 hours later.

In patients 3 and 4, after exposure to N₂O, more than 10% of DNA synthesis could be ascribed to ³H-thymidine after pre-incubation of the marrow with deoxyuridine. This relative lack of suppression was restored to normal by addition of either vitamin B₁₂ or folinic acid. Methyltetrahydrofolate also corrected the defect in patient 3. Patient 3 had an abnormal dUST before anaesthesia. In all other instances the dUST was within normal expected limits, i.e. less than 10% of the ³H-thymidine was used for DNA synthesis when the bone marrow had been incubated with deoxyuridine.

Bone marrow

In all patients the pre-operative and post-anaesthesia bone marrow was of normal cellularity and haemopoiesis was normal. In particular, there was no evidence of megaloblastic erythropoiesis and giant metamyelocytes and stell cells were absent.

TABLE III. PRE-OPERATIVE LABORATORY DATA

<table>
<thead>
<tr>
<th>Patient</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13,90 - 16,5</td>
</tr>
<tr>
<td>Leucocyte count (&lt;10⁹/dl)</td>
<td>12,39 - 14,0</td>
</tr>
<tr>
<td>Platelet count (&lt;10⁹/dl)</td>
<td>357,00 - 433,00</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87,20 - 95,00</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>2,28 - 2,50</td>
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<tr>
<td>Urea (mmol/l)</td>
<td>4,40 - 6,40</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>8,80 - 8,80</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>295,00 - 323,00</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>29,00 - 34,00</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>2,38 - 2,91</td>
</tr>
</tbody>
</table>
Biochemical profile

The serum albumin and cholesterol levels were reduced in all patients. This can probably be ascribed to malnutrition. Patients 2 and 4 showed increased levels of serum calcium, urea, creatinine and lactate dehydrogenase (LDH). Serum LDH levels were not elevated to the extent seen in megaloblastic anaemia (Table III).

Discussion

Amess et al.1 studied the effect of N2O in patients who had cardiac bypass surgery. They demonstrated that prolonged exposure (24 hours) to the gas induced megaloblastic haemopoiesis. These authors, utilising the dUST, correctly inferred that the N2O affected the metabolism of vitamin B12. N2O oxidises vitamin B12 in vitro from the cob(III)alamin to the inactive cob(II)alamin form thus blocking the availability of tetrahydrofolate required for the conversion of deoxyuridine to thymidine (Fig. 1). Vitamin B12 is the co-enzyme for methionine synthase and Deacon et al.5,6 proved that N2O rapidly inhibits the activity of the enzyme in the rat. Such inhibition, in both man and rat, soon interferes with DNA synthesis. This can be demonstrated by an abnormal dUST. Thus the dUST becomes abnormal in patients exposed to N2O before morphological changes become evident in the bone marrow. N2O anaesthesia was the cause of megaloblastic anaemia in severely ill patients admitted to an intensive care unit.4 In this study 18 of 22 patients had been exposed to N2O for only 2 - 6 hours. In these surgical patients with a variety of diseases (but no sepsis), there was a clear relationship between the degree of abnormalities of the dUST and the duration of N2O anaesthesia. Noteworthy was the finding that the dUST was more abnormal in those critically ill patients and its return to normal was slower compared with patients subjected to cardiac surgery. The mortality rate was also strikingly higher in those patients with megaloblastic bone marrow changes. In another study, a patient with severe haemorrhage had an abnormal dUST and megaloblastic marrow after exposure to N2O for only 1 hour.10

The anaemia of chronic disorders and inflammation is complex and the aetiology is multifactorial. Although the most important cause is considered to be a defect in haem synthesis,11 the anaemia may also be associated with depression of bone marrow function and a maturation arrest of the marrow precursors.12 Megaloblastic anaemia may also play a role in the development of anaemia in such patients, especially if they are very ill. Thus severe infection13,14 or fever15 may accelerate the development of folate deficiency in the critically ill. The role of vitamin B12 deficiency in these instances is not known. Shnier and Metz14 noted that some infants with laboratory evidence of folate deficiency and concomitant malnutrition may precipitate megaloblastic marrow changes.

Other factors may also contribute to the rapid development of megaloblastic anaemia in severely ill patients. Examples of these are intravenous feeding with amino acid-ethanol solutions16 and mild pre-existing vitamin B12 deficiency.17 It is evident from the foregoing that patients with severe infections exposed to even relatively short periods of N2O anaesthesia may, theoretically, be at risk. In patients 1, 2 and 5 there were no abnormalities of the dUST. The dUST of patient 3 was abnormal before and after exposure to N2O. These defects could be corrected by the addition of folinic acid to the deoxyuridine incubation medium. Patient 4 also had an abnormal dUST after exposure to N2O; this was corrected by folinic acid.

### TABLE IV. VITAMIN B12 AND FOLATE STATUS AND METABOLISM

<table>
<thead>
<tr>
<th>Patient</th>
<th>Vit B12 (ng/l)</th>
<th>Folate (µg/l)</th>
<th>Red cell folate (µg/l)</th>
<th>dUST (% of control tube)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Code</td>
</tr>
<tr>
<td>1</td>
<td>785</td>
<td>3</td>
<td>423</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>1307</td>
<td>2</td>
<td>263</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>251</td>
<td>7</td>
<td>ND</td>
<td>B</td>
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<td>4 &gt; 2000</td>
<td>5</td>
<td>569</td>
<td>A</td>
<td>10.6</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>7</td>
<td>493</td>
<td>B</td>
</tr>
</tbody>
</table>

Cod: B = before exposure to N2O; A = 24 h after exposure to N2O; A2 = 24 h after second laparotomy.

Normal serum values: Vitamin B12: 200 - 900 ng/l; folate: 3 - 20 µg/l; red cell folate: 160 - 640 µg/l. Du = deoxyuridine.

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**Fig. 1.** H2O oxidises vitamin B12 in vitro, and in vivo inhibits the activity of methionine synthase. These effects interfere with DNA synthesis. This may result in megaloblastic haemopoiesis.
Effect of desferrioxamine on reperfusion damage of rat heart mitochondria

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Summary

Ischaemia of the myocardium leads to necrosis unless oxygen supply is restored but it has only recently been realised that reperfusion is not without danger. The greatest rate of myocardial damage, as measured by mitochondrial function, occurred during the first 5 minutes of reperfusion in rat hearts subjected to normothermic ischaemic cardiac arrest. Addition of desferrioxamine to the perfusate after 5 minutes of reperfusion did not reverse the mitochondrial damage. It is therefore concluded that desferrioxamine prevents mitochondrial damage caused by ischaemia-reperfusion but does not reverse the damage already present.

REFERENCES


Necrosis is the ultimate outcome of myocardial ischaemia unless oxygen delivery is restored in time. However, reperfusion of ischaemic tissue is not without danger, in fact it has been shown that it adds insult to injury. Numerous circumstantial evidence suggests that this reperfusion injury — independent of ischaemic injury — is caused by the generation of oxygen-derived radicals, i.e. superoxide anions, hydrogen peroxide and hydroxyl radicals.

Oxygen-derived radicals are produced during the course of normal cell metabolism but are short-lived because of inactivation by protective mechanisms. These protective mechanisms are of two kinds: (i) enzymatic, e.g. superoxide dismutase, catalase and glutathione peroxidase; and (ii) non-enzymatic, e.g. vitamins C and E, and β-carotenes. These protective mechanisms have sufficient capacity to cope with the normal metabolic generation of oxygen-derived radicals. Superoxide radicals, if not immediately neutralised, can, through the release of iron from ferritin and other large proteins, generate the highly reactive hydroxyl radical via the Haber-Weiss and Fenton reactions.

The superoxide radicals implicated in ischaemia-reperfusion injury of the myocardium may have their origin in increased