

Haematological changes in a patient on long-term treatment with a trimethoprim-sulphonamide combination

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TRIMETHOPRIM (TMP) is an antibacterial chemotherapeutic agent of great interest as it is the first folic acid antagonist to be effective in the treatment for human bacterial infections. It is related to the antimalarial drug pyrimethamine, and similarly inhibits the enzyme dihydrofolate reductase. This enzyme is essential for the incorporation of folate into cell metabolism (see Fig. 1). There are species differ-

abnormalities in patients on high doses. The lower dose currently recommended for antibacterial therapy had not apparently caused any disturbance. This report concerns a patient who received the now conventional dose of TMP—320 mg daily—combined with sulphamethoxazole 1600 mg daily for 12 months, and who for much of that time had evidence of disordered folate metabolism.

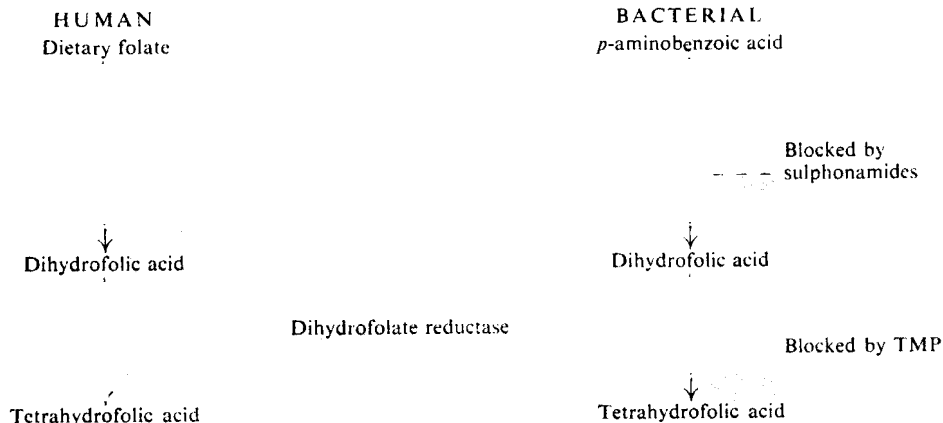


FIG. 1. Diagrammatic comparison of human and bacterial folate metabolism.

ences in the physical structure and perhaps the chemical form of dihydrofolate reductases. Exploitation of these differences has enabled the production of an inhibitor with 100,000 times greater affinity for bacterial dihydrofolate reductase than for the mammalian counterpart. At first TMP was thought to be without effect on folate metabolism in man, but Kahn, Fein & Brodsky (1968) showed some

Case report

The patient, a male Law Court attendant, was aged 59 when first admitted to hospital in 1965 with a chest infection. He had a 5 year history of productive cough. *Haemophilus influenzae* was constantly present in his sputum. He was treated with ampicillin and tetracycline with slow improvement over 2 months but the *H. influenzae* was only intermittently suppressed.

By November 1967 he was bedridden because of dyspnoea and *H. influenzae* was consistently present in his sputum in spite of treatment with various antibiotics. In December 1967 treatment was started with TMP and sulphonamide in combination. The result of this treatment was the rapid elimination of *H. influenzae* and no pathogen was isolated from his sputum on any occasion thereafter while he was on the drug.

In May 1968 he was transferred to our care.

Examination on admission. He was a wasted man weighing 60 kg and of 6 ft 2 in. height. He was extremely handicapped by dyspnoea but no longer bedridden, on occasion walking 10–20 yards. He was mildly cyanosed at rest with no clubbing of the fingers. He had signs of obstructive airways disease but no abnormality was detected in the cardio-vascular system, the abdomen, or the central nervous system. There was no evidence of salt and water retention.

The chest X-ray showed no focal lung lesions. The heart outline was normal but the main pulmonary artery and its branches were markedly enlarged. The ECG showed right axis deviation.

Laboratory investigation on admission. Hb 14.1 g/100 ml, PCV 45%, WBC 7500/mm³, neutrophils 58%, lymphocytes 31%, eosinophils 4%, monocytes 6%, basophils 1%, platelet count 152,000/mm³. Reticulocyte count 6% subsequently falling to 1.5%. The peripheral blood smear showed many macrocytes, numerous Howell-Jolly bodies and hypersegmented neutrophils.

An iliac crest bone marrow smear showed megaloblastic erythropoiesis and some giant metamyelocytes. Megakaryocytes were normal and stainable iron stores appeared decreased.

Red cell volume measured by dilution of ⁵¹Cr-tagged red cells was 40 ml/kg body weight (normal upper level 33 ml/kg (Dacie & Lewis, 1968)). Plasma volume by dilution of radioiodinated human serum albumin was 49 ml/kg body weight.

Serum iron by the method of Trinder (1956) was 175 µg/100 ml with total iron-binding capacity 348 µg/100 ml. Serum vitamin B₁₂, by the radioisotope dilution method of Raven, Walker & Barkhan (1966) 580 pg/ml. Radioactive vitamin B₁₂ absorption measured by the method of Harwood & Forshaw (1967) was normal as shown by a plasma level of 0.56% of dose per litre 10 hours after an oral dose. Serum folate levels by the method of Waters & Mollin (1961) using a trimethoprim-resistant mutant strain of *Lactobacillus casei* were 5.0 and 9.5 ng/ml. Red cell folate was 280 ng/ml (normal range 165–650 ng/ml).

The plasma levels of sodium, potassium, chloride and bicarbonate were 128, 3.4, 93 and 35 mEq/l

respectively, plasma urea 34 mg/100 ml, arterial blood pH 7.39 and PCO₂ 49 mmHg.

Further investigation and progress

The patient was receiving four standard tablets of the TMP-sulphonamide combination daily totalling trimethoprim 320 mg with sulphamethoxazole 1600 mg daily. He was also receiving betamethasone 0.25 mg twice daily, frusemide 40 mg daily with potassium supplements, methandienone 5 mg daily, probanthine 15 mg daily and orciprenaline bronchodilator spray. This regimen was continued unaltered except that the diuretic dose was varied as part of a study of the mechanism of his dyspnoea.

Until the connection between the abnormal blood picture and TMP was proved and its seriousness assessed, it was not felt justifiable to stop this drug, from which he had received such obvious benefit. The patient agreed to undergo some investigations to settle the matter.

The normal serum and red cell folate levels seemed to exclude simple folate deficiency due to inadequate diet or intestinal malabsorption. However folic acid 50 µg was given daily by intramuscular injection for 14 days, then 100 µg daily for a further 10 days, after which the bone marrow picture was unchanged. During this period there had been no change in the peripheral blood picture.

In view of the mode of action of TMP, folic acid was substituted for folic acid.

Folic acid 60 µg was given daily by injection for 7 days, after which bone marrow examination showed erythropoiesis to be entirely normoblastic. No giant metamyelocytes were now seen.

Folic acid was now continued in this dosage for a total of 18 days after which the bone marrow findings were again confirmed. Subsequently the number of macrocytes in the peripheral blood diminished, and the reticulocyte count rose slightly from 1.5% to 5%. There was no change in the white cell total or differential counts.

During the first month following the discontinuation of folic acid, the peripheral blood film did not change except for a slight decline in the reticulocyte count. At the end of this time the bone marrow was still normoblastic. The red cell volume was found to have dropped to 28.5 ml/kg body weight.

During the second month after stopping folic acid the peripheral haemoglobin concentration fell from 13.3 g/100 ml to 10.5 g/100 ml. This was partly due to haemodilution following relaxation of his diuretic regime. However, his red cells were becoming increasingly hypochromic and macrocytes reappeared. The red cell volume declined to 24.7 ml/kg body weight but at the end of this period a further bone marrow examination was still normal except for reduced iron stores.

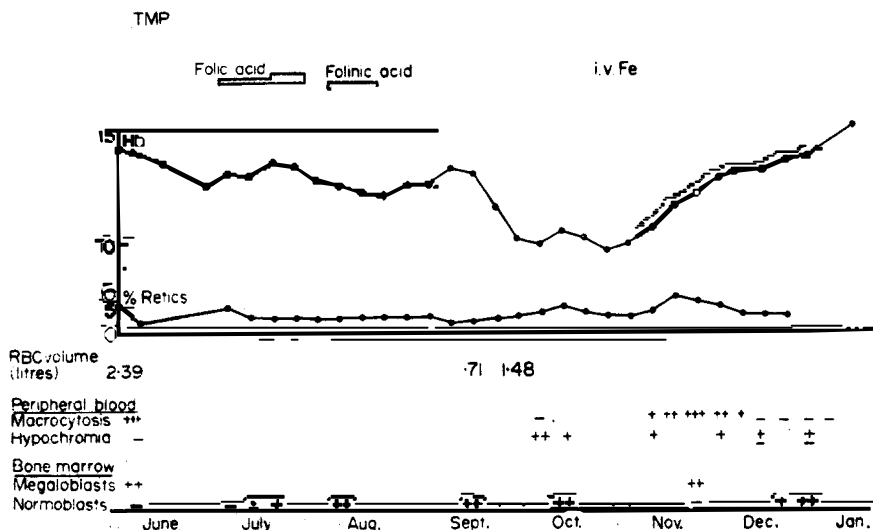


Fig. 2. Findings

In the third month after stopping folinic acid the patient received 1100 mg of iron as iron dextran (Imferon) intravenously and there followed a rise in haemoglobin concentration to 12.9 g/100 ml with a slight rise in reticulocyte count to 7%. The white cell total and differential count remained unaltered and within normal limits throughout. The platelet count did not fall below 248,000/mm³. Three and a half months after stopping folinic acid the megaloblastic picture of the bone marrow had reappeared.

At this stage it was judged safe to discontinue TMP. He had received it for nearly 12 months and during that time there had been no evidence of bronchial infection. Sulphamethoxazole was continued in the dosage that he had been receiving in the combined tablet. Fifteen days after discontinuing TMP, erythropoiesis was almost completely normoblastic. After a further 10 days, the patient was generally less well and scanty growths of coagulase-positive *Staphylococci* were obtained intermittently from his sputum. As this organism was sensitive to TMP it was thought that it was no longer justified to withhold the drug. A final bone marrow examination 25 days after stopping TMP confirmed the improvement seen 10 days before.

Some weeks later the patient was subjected to a right lower lobectomy in an attempt to improve his ventilation by reducing dead space. The operation was uneventful but 10 days later the patient developed a *Pseudomonas pyocyanea* chest infection to which he rapidly succumbed. Necropsy showed abnormalities confined to the lungs and heart. The lungs showed the changes of chronic bronchitis and emphysema with extensive pneumonia. The heart showed right ventricular hypertrophy of moderate degree.

Discussion

This patient received great benefit from TMP as a long-term agent to suppress his bronchial infection. It was effective in eradicating all obvious pathogens from his sputum for more than a year. He was aware of no side-effects and the defect in folate metabolism was not associated with any observable ill-health.

After 6 months of the TMP-sulphonamide combination his peripheral blood examination showed definite abnormality of red and white cell morphology but with a normal platelet count. Bone marrow examination showed megaloblastic change. The packed cell volume was normal but the red cell volume was above normal. This erythrocytosis was masked by the coexistent large plasma volume, resulting in a normal venous haematocrit. It was presumably due to his reduced arterial oxygen saturation from his pulmonary disease.

Vitamin B₁₂ deficiency was excluded, as was simple folate deficiency due to poor diet or malabsorption or as commonly seen during any severe prolonged illness. The marrow would certainly revert to normal after nearly 2 mg of folic acid by injection over 4 weeks. The ineffectiveness of this treatment may be contrasted with the complete response to less than 500 µg of folinic acid over 7 days. This is strong evidence in favour of the existence of a block in folate metabolism at the level of dihydrofolate reductase.

This patient received 60 µg per day of folinic acid for 18 days. The daily requirement of folinic acid by a normal subject is not known but this is of the order of the normal daily requirement of folic acid (Herbert, 1964). Cessation of treatment was not associated with an immediate relapse into megaloblastic erythropoiesis. The peripheral blood, however, showed

increasing evidence of iron deficiency. Though the red cell volume was still within the normal range, it declined by 38% from the original measurement. This decline was probably due to the interaction of several factors. Most important was iron deficiency, doubtless aggravated by the frequent (usually daily) blood sampling as part of this patient's intensive respiratory investigation. A second factor could be the overall tendency for this patient's arterial oxygen tension to rise during his stay—amounting on average to an increase of 10 mmHg. The defect in folate metabolism is unlikely to be the explanation as the bone marrow was normal for most of this time.

Following correction of the iron deficiency the florid picture of folate deficiency reappeared.

In view of the rapidity and completeness of the response to folinic acid, the prompt reversion to normality on stopping TMP was not surprising. The stress of the recrudescence of his chest infection did not cause a relapse in the absence of TMP.

There is only one other published report suggesting disturbed folate metabolism in man during treatment with TMP. Kahn *et al.* (1968) have studied the effects of a high dose of TMP—sulphonamide combination (TMP 1000 mg and sulphamethoxazole 4000 mg daily). In a short-term study of ten subjects treated for 28 days, all cases developed changes in the morphology of the granulocytes of the peripheral blood with increased lobulation of nuclei. Five subjects developed bone marrow changes described as 'transitional megaloblastic erythroid change' and three others showed abnormalities of white cell precursors with giant metamyelocytes. One case had anaemia and one thrombocytopenia.

In their long-term studies of thirteen subjects given the same dose for 6–12 months, they found evidence of haematologic abnormality in six cases by examination of the peripheral blood but no bone

marrow examinations were done. One of the six became anaemic and the other five had changes confined to the white cells or the platelet count. In one of these subjects they succeeded in reversing the peripheral blood change with folinic acid while TMP was continued, but they formed the impression that definitely 'supra normal' quantities were needed.

The currently recommended dose of TMP is only one third of that used in the above study by Kahn *et al.* (1968). Our patient received the drug as currently recommended and is the first reported case of haematological abnormality with this dose.

Acknowledgments

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