glycaemia, and the present trial refers to patients initially treated in this way. Yet, even in these patients, when oral therapy was discontinued 31% remained as well controlled as when taking their tablets. These findings suggest that even when the need for tablet therapy has been established by a poor initial response to simple dietary restriction, the introduction of tablets should not be regarded as necessarily permanent. The dose can steadily be reduced and then discontinued if normoglycaemia is maintained. When hyperglycaemia recurs it seems reasonable, in the present state of our knowledge, to reintroduce tablet therapy at the previous effective dose level. There is no firm evidence that insulin is more or less effective than oral therapy in preventing the degenerative complications in diabetics who cannot be controlled on simple dietary restriction.

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Toxicity of Trimethoprim-Sulphamethoxazole in Patients with Megaloblastic Haemopoiesis

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Summary

Four consecutive patients with megaloblastic anaemia who also received therapy with trimethoprim-sulphamethoxazole all showed poor responses to specific haematinic therapy. This was attributed to trimethoprim, which suppressed reticulocyte responses in three cases and produced a pancytopenia in two and a falling haemoglobin with neutropenia in a third. A fourth patient, with pernicious anaemia, had a satisfactory reticulocyte response but experienced no clinical benefit until after withdrawal of trimethoprim.

Trimethoprim seems not to be a safe form of therapy in patients with a megaloblastic process; many of the toxic reactions reported with this drug may be on the basis of an unrecognized megaloblastic form of haemopoiesis.

Introduction

The trimethoprim-sulphamethoxazole drug combination has been in clinical use since 1968 and relatively few serious haematological complications associated with its administration have been recorded.

Whitman (1969) found that 1 g of trimethoprim daily given to healthy volunteers produced megaloblastic marrow changes in 8 out of 10 subjects. A similar dose given to 13 patients with chronic urinary tract infection produced some evidence of haematological change in 11—that is, leucopenia, thrombocytopenia, or appearance of hypersegmented neutrophils (Kahn, Fein, and Brodsky, 1968).

In the first six months after the introduction of the drug combination Hanley (1969) collected four examples of depression of leucocytes, nine of thrombocytopenia or purpura, and one report of aplastic anaemia. Allison, Kennedy, Mc-

Geachie, and McDonald (1969) did not find any appreciable blood changes in 25 women treated for seven days, although there was a significant fall in serum folate level. Hughes (1969) did not record any blood changes in 25 chronic bronchitics treated for seven days.

One patient with thrombocytopenia and megaloblastic marrow changes among 10 chronic bronchitics given 250 mg of trimethoprim and 500 mg of sulphamethoxazole twice daily for three months was found by Jenkins, Hughes, and Hall (1970). A further case of megaloblastic anaemia with this drug combination was reported by Jewkes, Edwards, and Grant (1970). Anaemia, neutropenia, and thrombocytopenia in a puerperal woman given trimethoprim-sulphamethoxazole was reported by Mohan (1969). Other examples of neutropenia were recorded by Pauley (1970), Evans and Tell (1969), McCarthy (1969), and Hulme and Reeves (1971). Three cases of thrombocytopenia were recorded by McPherson and Raik (1970) and Hammett (1970) and a fourth patient had pancytopenia with megaloblastic marrow.

Scragg and Rubidge (1971) treated African and Indian children with typhoid fever with trimethoprim and sulphamethoxazole for 21 days. Neutropenia was noted twice as often as in a chloramphenicol-treated control group. Two children with neutropenia were also noted to have a reticulocytosis after withdrawal of therapy. Dawson and Routledge (1971) reported the appearance of a megaloblastic marrow in a woman given trimethoprim-sulphamethoxazole in whom the red cell folate level was only 16 ng/ml.

Urinary tract infection is a frequent complication in women with megaloblastic anaemia, and in the past we have used trimethoprim-sulphamethoxazole as the drug of choice. The purpose of this paper is to draw attention to the toxic effects of this drug when given to patients who also have a megaloblastic process and to describe the clinical and haematological changes in four consecutive patients so treated.

Case Reports

CASE 1. NUTRITIONAL VITAMIN B₁₂ DEFICIENCY

The patient, a 21-year-old Indian woman from Kenya, complained of a sore tongue for four weeks. She was a strict vegetarian. The haemoglobin concentration was 6.1 g/100 ml, total white cell

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count was 5,100/mm³ (neutrophils 2,650), and platelets were 110,000/mm³. The blood film was macrocytic with hypersegmented neutrophils and the marrow was megaloblastic. Serum vitamin B₁₂ level was 65 pg/ml, serum folate 3 ng/ml, and red cell folate 200 ng/ml. The gastric juice after pentagastrin had a pH of 1.9 and an intrinsic factor output of 2,300 units in one hour. Faecal fat excretion, xylose excretion, and barium-meal appearances of the intestinal tract were normal. She absorbed 42% of an oral 1-μg dose of ⁵⁷Co-B₁₂.

A diagnosis of nutritional vitamin B₁₂ deficiency was made and treatment with 2 μg of vitamin B₁₂ daily by mouth was started. A routine midstream urine sample was found to contain over 10⁵ *Escherichia coli* organisms/ml, which were sensitive to trimethoprim-sulphamethoxazole, and treatment with this drug combination (Bactrim)—trimethoprim 320 mg and sulphamethoxazole 1,600 mg daily—for 10 days began on day 2 after starting oral vitamin B₁₂.

The haematological changes are shown in Fig. 1. The reticulocytes reached 10% on day 6 after starting vitamin B₁₂ but thereafter they declined, and this was accompanied by a fall in the neutrophil

FIG. 1—Haematological changes and therapy in a patient with nutritional vitamin B₁₂ deficiency (Case 1).

count from 2,000 cells/mm³ to 650/mm³ and by a fall in the platelet count from 190,000/mm³ to 85,000/mm³. There was no clinical benefit from vitamin B₁₂ therapy and she continued to feel unwell until trimethoprim was withdrawn.

After trimethoprim was stopped there was a second reticulocytosis, reaching 24.6%, and a rise in neutrophils, platelets, haemoglobin, and red cell count. This was accompanied by appreciable clinical improvement, with return of appetite and energy and disappearance of the tongue soreness.

CASE 2. NUTRITIONAL FOLATE DEFICIENCY

This 76-year-old woman was referred by her general practitioner because of persistent anaemia. There was some degree of dementia. The haemoglobin was 9.6 g/100 ml, white cell count was 5,600/mm³ (neutrophils 4,600), and platelets were 50,000/mm³. The stained blood film was macrocytic and the marrow showed megaloblastic haemopoiesis. After pentagastrin a small volume of gastric juice of pH 7.6 was yielded, and the intrinsic factor was not measurable. The fasting serum gastrin level (800 units/ml) was raised. A provisional diagnosis of pernicious anaemia was made and treatment with intramuscular injections of vitamin B₁₂ (2 μg daily) was started. A urinary tract infection was also present, and trimethoprim (320 mg) and sulphamethoxazole (1,600 mg) daily were started on the second day after vitamin B₁₂.

The haematological changes are shown in Fig. 2. There was a poor reticulocyte response. The serum vitamin B₁₂ level (320 pg/ml), which became available at this point, indicated that the patient did not, in fact, have vitamin B₁₂ deficiency. Further, the low

serum folate (1.4 ng/ml) and red cell folate (53 ng/ml) indicated folate deficiency. Subsequently faecal fat excretion, xylose excretion, barium meal and follow-through examination, and a normal vitamin B₁₂ absorption (uptake of 61% of a 1-μg dose of ⁵⁷Co-B₁₂ with normal plasma radioactivity at 10 hours) suggested a diagnosis of nutritional folate deficiency.

FIG. 2—Haematological changes and therapy in a patient with nutritional folate deficiency (Case 2).

Vitamin B₁₂ injections were stopped and the patient remained on Bactrim pending the completion of intestinal function studies. During this period the platelets declined to 13,000/mm³, neutrophils to 450/mm³, reticulocytes disappeared, and there was a steady fall in haemoglobin to 7.5 g/100 ml. Purpura appeared on both legs. The marrow, which initially had shown a moderate degree of megaloblastic change, showed severe megaloblastic changes with an accumulation of basophilic and pro-megaloblasts. Large oral doses of folic acid were given (5 mg daily) as well as an injection of folic acid (3 mg) (Fig. 2). Within 48 hours of folate therapy there was a reversion of the marrow to largely normoblastic haemopoiesis, and this was followed by a return of neutrophil and platelet levels to normal. There was a slower recovery of the haemoglobin level.

Assay of the leucocytes after recovery did not produce measurable levels of the enzyme dihydrofolate reductase.

CASE 3. FOLATE DEFICIENCY AND RHEUMATOID ARTHRITIS

The patient, a 56-year-old woman with an eight-year history of rheumatoid arthritis, was admitted to hospital with infected ulcers on the left leg and both feet. The E.S.R. was 143 mm in one hour and the latex test for rheumatoid factor was strongly positive. Although the joint manifestations appeared to be quiescent she was thought to have active vasculitis.

The haemoglobin concentration was 8.6 g/100 ml, total white cell count was 5,500/mm³ (neutrophils 3,000), and platelets were 300,000/mm³. The blood film was macrocytic and the marrow showed megaloblastic haemopoiesis. There was no stainable iron in the marrow. Swabs from the leg ulcers yielded growths of *Staphylococcus aureus* and *Streptococcus faecalis*, which were sensitive to penicillin and trimethoprim. Therapy with penicillin was started. A generalized rash appeared after five days and the treatment was therefore changed to trimethoprim (320 mg) and sulphamethoxazole (1,600 mg).

The megaloblastic process was due to folate deficiency. The serum vitamin B₁₂ level was equivocal (150 pg/ml) but she absorbed vitamin B₁₂ normally (74% of a 1-μg dose of ⁵⁷Co-B₁₂ by the faecal excretion method). The serum folate was low (1.0 ng/ml) but red cell folate was still normal (210 ng/ml). There was acid in the gastric juice (pH 2.1 after pentagastrin) and she secreted 680 units of intrinsic factor in an hour. Folic acid 200 μg daily by mouth was started on the sixth day after the start of trimethoprim.

The haematological changes are shown in Fig. 3. There was a steady decline in haemoglobin, red cell count, platelets, and neutrophils. The response to folic acid was delayed, reticulocytes reaching 16% on the ninth day after the start of folate, and after

trimethoprim was withdrawn a second peak of 12% occurred. Thereafter there was a rise of the red cells, platelets, and neutrophils.

FIG. 3—Haematological changes and therapy in a patient with folate deficiency associated with rheumatoid arthritis (Case 3).

CASE 4. PERNICIOUS ANAEMIA

This 81-year-old woman was admitted to hospital with a haemoglobin concentration of 7.5 g/100 ml and a megaloblastic marrow. Her serum vitamin B₁₂ level was 60 pg/ml and she had a pentagastrin-fast achlorhydria with only 60 intrinsic factor units in the hour. Tests of vitamin B₁₂ absorption by the urinary excretion method gave an excretion of 0.8% with the dose of vitamin B₁₂ alone and 24.6% when it was repeated with additional intrinsic factor.

Although there were no symptoms referable to the urinary tract urine culture showed a heavy growth of *E. coli*. Treatment with trimethoprim (320 mg) and sulphamethoxazole (1,600 mg) was started three days after she had received an injection of 1,000 μg of vitamin B₁₂. The haematological response after vitamin B₁₂ therapy appeared adequate (21% reticulocytes on day 6 with a rise in red cell count) but she did not experience any clinical benefit—that is, there was no improvement in well-being, energy, and appetite that is normally such a characteristic feature of the response in pernicious anaemia. These features appeared only two to four days after the course of trimethoprim had terminated.

Discussion

These observations in patients with megaloblastic anaemia who were treated with trimethoprim-sulphamethoxazole show that this drug combination frequently produces a failure of haemopoiesis. In particular thrombocytopenia and neutropenia were prominent features in two of the four cases described. If given sufficiently early in relation to the haematinic substance (Case 1)

it prevented the expected reticulocyte response to specific therapy. When given later (Case 4) the haematological response was adequate but there was no clinical benefit as a result of treatment.

A megaloblastic form of haemopoiesis is commonplace in clinical practice. Thus some 25% of healthy pregnant women show megaloblastic marrow changes near term (Chanarin, 1969), as do 17% of patients on anticonvulsant drugs. Minor increases in red cell volume are common when automatic blood counting machines of the Coulter S type are used, and usually indicate megaloblastic marrow changes, although haemoglobin levels are often still within normal limits. Low serum folate levels are found in one-third of patients requiring hospital admission (Chanarin, 1969). Further, the frequency of pernicious anaemia in the U.K. in subjects over the age of 60 approaches 1% (Chanarin, 1969).

It seems likely that all such patients with early megaloblastic changes are at risk from trimethoprim, and the occurrence of thrombocytopenia and neutropenia in patients given this drug combination occasionally reported in the literature probably arises on the basis of a megaloblastic process.

We have no evidence that trimethoprim rather than sulphamethoxazole was responsible for the blood changes in the present patients. However, such frequent blood changes have not hitherto been seen with sulphonamides, and it seems probable that trimethoprim is the toxic agent.

Trimethoprim has a methotrexate-like effect in bacterial systems—that is, it links to the enzyme dihydrofolate reductase. The evidence that it has a similar action on the mammalian enzyme is poor, and Dr. K. Rowe in our laboratory has failed to show any effect on dihydrofolate reductase from the rat or human fetal liver. In man exposure to methotrexate leads to progressively higher dihydrofolate reductase levels in leucocytes and other tissues. It is significant that we failed to show such a rise in Case 2. Thus we feel that the mode of toxic action of trimethoprim in man is yet to be determined.

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