Immunological effect of co-trimoxazole on platelets

FRANS H J CLAAS, JOS W M VAN DER MEER, JANNEKE LANGERAK

Summary and conclusions
Diminished survival of transfused platelets occurred in two patients given co-trimoxazole, and a third patient taking this drug developed thrombocytopenia. By means of an indirect immunofluorescence assay antibodies against donor platelets coated with co-trimoxazole were found in the sera in all cases. These antibodies were directed against the trimethoprim component of co-trimoxazole and not against sulphamethoxazole. Co-trimoxazole is a potent antimicrobial agent and is advocated for treatment and prophylaxis in leukaemia. Hence its adverse effect on platelets is of great importance.

Introduction
Co-trimoxazole (trimethoprim-sulphamethoxazole), a potent antimicrobial drug, is effective in various infections. The antimicrobial activity is due to sequential blockade of the synthesis of folic acid: sulphamethoxazole competes with para-aminobenzoic acid in the synthesis of dihydrofolic acid, and trimethoprim inhibits dihydrofolate reductase, the enzyme that converts dihydrofolate to tetrahydrofolate. Reported haematological effects of co-trimoxazole mostly concern megaloblastic transformation, which probably results from the effect of trimethoprim on dihydrofolate reductase. It is not clear, however, whether thrombocytopenia or neutropenia or both in the absence of megaloblastic changes are also due to trimethoprim or caused by the sulphamethoxazole component.

Two patients receiving platelet transfusions showed diminished survival of transfused platelets during treatment with co-trimoxazole, and a third patient taking the drug developed thrombocytopenia and neutropenia. We therefore used an indirect immunofluorescence assay to test sera from these three patients against donor platelets coated with trimethoprim, sulphamethoxazole, and the combined agent.

Subjects and methods

PATIENTS AND CONTROLS
Case 1—A 43-year-old man, suffering a blast crisis of chronic myelogenous leukaemia was treated with aggressive chemotherapy (daunorubicin, cytarabine, thioguanine, and cyclophosphamide) and total body irradiation, and given platelet transfusions because of thrombocytopenia. He then began a nine-day course of co-trimoxazole (2 tablets twice daily) for a bacterial infection. On day 3 of this treatment poor survival of transfused platelets was observed. When the course ended survival of transfused platelets became normal.

Case 2—A 20-year-old man received a bone marrow transplant for severe aplastic anaemia, and because of deep thrombocytopenia he was given platelet transfusions. The platelets survived poorly during two periods of six days in which he received co-trimoxazole (2 tablets twice daily) for a staphylococcal infection. After stopping the drug survival of the donor platelets became normal.

Case 3—A 52-year-old woman was being treated for staphylococcal spondylodiscitis. Because of penicillin allergy (fever and neutropenia) co-trimoxazole 3 tablets twice daily was instituted. On about the 10th day of treatment neutropenia (20 × 10⁹ granulocytes/l (20 mm³)) and thrombocytopenia (100 × 10⁹ platelets/l (100 000 mm³)) were found. After withdrawing co-trimoxazole there was a slow recovery.

Controls—Five hospital inpatients with normal platelet counts taking co-trimoxazole for various infections and 10 healthy blood donors served as controls.

METHODS
Platelet survival—Usually preparations from four different donors containing an average of 150 × 10⁹ platelets were transfused. In the absence of immunological destruction this would be expected to increase the circulating platelet count by 20-30 × 10⁹/l (20 000-30 000/mm³) one hour after transfusion (in an adult of about 70 kg). Survival of transfused platelets was regarded as poor when the increase was less than 10 × 10⁹/l.

Isolation of platelets—EDTA blood (1 part 5%, sodium EDTA and 9 parts blood) was centrifuged for 30 minutes at 150 g. The upper layer of plasma containing the platelets was washed three times with phosphate-buffered saline containing 0·3%, sodium EDTA, and a 1 × 10⁸/ml suspension was made in this same medium.

Indirect immunofluorescence—Sera from the patients and controls were tested against platelets from healthy donors. Two drops of platelet suspension were tested against platelets from the same donor.

Incubation of platelets with antimicrobial agent—Platelets were incubated for 15 minutes at room temperature with 0·1 ml co-trimoxazole (16 mg trimethoprim and 80 mg sulphamethoxazole/ml, Wellcome-Nederland BV, Amsterdam), 0·1 ml trimethoprim (20 mg/ml, Wellcome-Nederland BV, Amsterdam), and 0·1 ml sulphamethoxazole (80 mg/ml, Hoffmann La Roche, Mijdrecht). After washing, the platelets were incubated with the test sera as described for the untreated platelets. The reactions of platelets incubated with each agent were compared with untreated platelets from the same donor.

Results
In the indirect immunofluorescence tests sera from all three patients were found to contain antibodies against platelets incubated with co-trimoxazole. These antibodies were directed against the trimethoprim component only (table).

During co-trimoxazole treatment in case 2 the patient's platelets were tested by direct and indirect immunofluorescence with his own serum; both tests were positive regardless of whether the platelets had been incubated with co-trimoxazole. Four weeks after stopping co-trimoxazole direct immunofluorescence on the platelets was negative with and without co-trimoxazole incubation. Nevertheless, when the platelets were incubated with co-trimoxazole the indirect immunofluorescence test became positive, indicating that the antibody was still present in his serum.

Department of Immunohaematology, University Medical Centre, Leiden, Netherlands
FRANS H J CLAAS, MB, research fellow
JANNEKE LANGERAK, technician

Department of Infectious Diseases, University Medical Centre, Leiden, Netherlands
JOS W M VAN DER MEER, MD, consultant in infectious diseases
Drug-induced immune thrombocytopenia has been shown for several other drugs. The best known examples are quinine and quinidine,\textsuperscript{1,2} but allylisoprocyclacarbamide\textsuperscript{3}; sulphafurazol, diazepam, and phenytoin\textsuperscript{4}, and rifampicin\textsuperscript{5} have also been implicated. Why some patients develop these antibodies and others do not remains a subject for further study.

We thank Dr H L Haak and Dr J Nauta for their co-operation, Dr J S Thompson and Professor Dr R van Furth for critically reading the manuscript, and Ms Jeanne van Nassau for preparing the manuscript.

This study was in part supported by the National Institutes of Health (contract N01-AI-82553); the Dutch Organisation for Health Research (FUNGO); the Dutch Foundation for Medical Research (ZWO); and the J A Cohen Institute for Radiopatology and Radiation Protection.

References
\textsuperscript{1} Bushby, S, R M, and Hitchings, G H, British Journal of Pharmacology, 1968, 72.
\textsuperscript{2} Salter, A J, Medical Journal of Australia, 1973, Suppl No 70.
\textsuperscript{3} Frisch, J M, Journal of Infectious Diseases, 1973, 128, Suppl No 175.
\textsuperscript{4} Ferone, R, Burchall, J J, and Hitchings, G H, Molecular Pharmacology, 1968, 5, 49.
\textsuperscript{5} Australian Drug Evaluation Committee, Medical Journal of Australia, 1972, 1, 435.
\textsuperscript{8} Hughes, W T, New England Journal of Medicine, 1977, 297, 138.
\textsuperscript{11} Shulman, N R, Journal of Experimental Medicine, 1958, 107, 711.
\textsuperscript{12} Karpatkin, S, American Journal of Medical Sciences, 1971, 262, 69.
\textsuperscript{13} Ackroyd, J F, Clinical Science, 1949, 7, 249.

(Accepted 7 August 1978)