Sulfadoxine specific lymphocyte transformation in a patient with eosinophilic pneumonia induced by sulfadoxine-pyrimethamine (Fansidar)

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ABSTRACT A patient developed eosinophilic peripheral pulmonary infiltrates while receiving malaria prophylaxis with sulfadoxine-pyrimethamine (Fansidar). Withdrawal of Fansidar and treatment with corticosteroids led to rapid recovery. No exacerbation occurred after cessation of corticosteroids. Lymphocyte transformation testing gave a positive result in the presence of sulfadoxine but not pyrimethamine. It is concluded that drug hypersensitivity to sulfadoxine was the cause of the eosinophilic pneumonia in this patient.

Pyrimethamine-sulfadoxine (Fansidar) has been used extensively worldwide for malaria prophylaxis in areas endemic for chloroquine resistant *Plasmodium falciparum* malaria. Although several sulphonamides have been reported to cause eosinophilic pneumonia and other hypersensitivity reactions, pulmonary lesions are a rare complication of treatment with Fansidar.¹ Only three cases of Fansidar induced eosinophilic pneumonia have been published²⁻⁴ and 11 cases of adverse drug reactions with pulmonary manifestations are known to the manufacturer (personal communication, Hoffmann-LaRoche, Grenzach-Wyhlen, West Germany).

Methods

The lymphocyte transformation test was performed as described.^{5,6} In brief, peripheral blood mononuclear cells from the patient and a healthy donor (who had never taken Fansidar) were isolated from heparinised blood by standard Ficoll-Hypaque density gradient centrifugation. The cells were aspirated from the interface, washed three times in Hanks's balanced salt solution, and resuspended in RPMI 1640 (Flow) supplemented with glutamine. Viability assessed by staining with acridine orange was over 95%; contamination with polymorphs was under 5%. The mononuclear cells (3×10^5 per well) were cultured in hexaplicate for six days in 200 μ l RPMI 1640 supplemented with glutamine and 20%

heat inactivated pooled human AB serum in the presence of 0.1–100 μ g/ml sulfadoxine or pyrimethamine. Alternatively, the mononuclear cells were incubated in 200 μ l RPMI 1640 supplemented with 20% heat inactivated serum containing drug metabolites (see below). Eighteen hours before being harvested, the cultures were pulsed with 1 μ Ci tritiated thymidine. Bound radioactivity was measured by liquid scintillation counting. Data are presented in terms of the stimulation index (SI), calculated by dividing the activity in counts per minute (cpm) of cultures with drug or serum containing metabolites by that of the cultures containing neither drug dilutions nor serum containing metabolites. The mean activity of control cultures (for example, SI = 1) in RPMI supplemented with pooled AB serum was 1200 (healthy donor) and 1700 cpm (patients); the means for control cultures in RPMI supplemented with serum obtained before ingestion of the drug were 1300 cpm (healthy donor) and 1350 cpm (patient) (the standard deviation was less than 10%). Statistical significance was calculated by Student's *t* test.

Sera containing "metabolites" were obtained from two healthy volunteers, one of whom ingested a single dose of 100 mg pyrimethamine and the other a single dose of 1000 mg sulphadoxine. Blood was drawn before (*t* = 0) and after ingestion of the drug at intervals up to four hours. The blood was allowed to clot and was centrifuged at 1000 *g* for 10 minutes. The serum was filtered through 0.22 μ m filters (Millipore), heat activated (30 min at 56°C), and centrifuged at 2000 *g* for 20 minutes. No further steps were taken to characterise further the sulfadoxine or pyrimethamine metabolites.

Case report

A 42 year old woman travelled to south-east Asia on 3 May 1985. One day before her departure she started malaria prophylaxis with Fansidar (25 mg pyrimethamine, 500 mg sulfadoxine per week) and this was continued until 28 June (in total nine doses). On 17 May she developed a cough, which worsened progressively and became productive with purulent sputum, and which was associated with dyspnoea at rest, and fever up to 38.8°C. After her return home at the end of May her chest radiograph showed peripheral infiltrates and treatment with ampicillin was started. Her symptoms worsened and the patient was admitted to hospital on 14 June.

Physical examination showed no lung abnormalities.

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Fig 1 Posteroanterior chest radiographs taken (a) when the patient was admitted, with bilateral patchy peripheral infiltrates ('photographic negative of pulmonary oedema'¹²); and (b) after 14 days' steroid treatment, showing resolution of the peripheral opacities.

There was no rash, arthralgia, or lymphadenopathy. Fever persisted (38.6°C) and chest radiographs showed increased peripheral patchy confluent infiltrates with a minor pleural effusion on the right (fig 1a). Laboratory examination showed a rise in the erythrocyte sedimentation rate to 140 mm in the first hour (Westergren); 8.4×10^9 leucocytes/l with 14% eosinophils, 51% neutrophils, 33% lymphocytes, 2% monocytes; 504×10^9 thrombocytes/l; and a haemoglobin concentration of 9.6 g/dl. Serum electrolytes and bilirubin concentrations, transaminase activities, and the results of coagulation tests were normal. Total plasma

proteins were 67 g/l with 17% α_2 and 18% γ globulins. The arterial oxygen tension (PaO_2) was 8.8 and carbon dioxide tension (PaCO_2) 4.5 kPa. Microbiological sputum and blood examination and tests for autoantibodies gave negative results.

As erythromycin treatment (1.5 g/d) initiated four days after admission to hospital failed and transbronchial lung biopsy showed non-specific changes, a diagnostic thoracotomy was carried out. Histological examination showed alveolar pneumonitis and interstitial inflammatory infiltration with lymphocytes, eosinophils, and few plasma cells. The alveoli were filled with dense masses consisting of macrophages and eosinophils displaced partially by granulation and fibrotic tissue. Interstitium and walls of the small bronchioli contained follicle like lymphocyte agglomerations. There was no evidence of granulomatous vasculitis, neoplasia, or parasites.

As these diagnostic procedures did not allow a firm diagnosis, the possibility of a drug hypersensitivity reaction was considered. Fansidar and erythromycin were withdrawn and treatment with fluocortolon 100 mg/day was initiated. This led to a rapid fall of fever and relief of cough and dyspnoea and the radiographic infiltrates decreased (fig 1b). Fluocortolon was rapidly tapered to a dose of 20 mg/day and the patient was discharged on 17 July. At an outpatient visit two weeks later PaO_2 was normal at rest and pulmonary function tests showed a minor restrictive ventilatory defect.

Lymphocyte transformation tests⁵⁻⁷ were performed at the time of discharge—that is, 20 days after drug withdrawal—and showed appreciable stimulation of the patient's lymphocytes in the presence of sulfadoxine (fig 2). In contrast, pyrimethamine and its metabolites had no stimulatory effect. Lymphocytes from a healthy donor (fig 2) were not stimulated. Neither the two drugs nor the corresponding serum samples containing metabolites stimulated proliferation of lymphocytes obtained from 12 healthy donors tested during the last three years as controls in other cases of suspected hypersensitivity to Fansidar.

Discussion

The term eosinophilic pneumonia is widely used to classify a syndrome of pulmonary infiltrations with eosinophils and histiocytes that is frequently accompanied by peripheral blood eosinophilia. The clinical course may vary considerably from uncomplicated transitory infiltrations (Löfller syndrome⁸) to severe manifestations with a potentially fatal outcome. The aetiology is often unknown, although a hypersensitivity reaction against parasite antigens or drug haptens has been discussed.⁹

The immune mechanisms in eosinophilic pneumonia are still not clear. T cell derived cytokines (for example, interleukin-5 (IL-5) and IL-3) have been shown to stimulate eosinophil growth and differentiation, and platelet activating factor is chemotactic for eosinophils.¹⁰ Thus stimulation of T cells and release of platelet activating factor by mast cells may induce the observed local recruitment of eosinophils. Activation of eosinophils could then result in the synthesis of reactive oxygen radicals and the release of cytotoxic mediators, such as major basic protein, which may be responsible for the observed lesions.¹¹

In our patient cessation of Fansidar and steroid treatment

Fig 2 Results of lymphocyte transformation tests performed with lymphocytes from the patient (solid symbols) and a control subject (open symbols). Results are expressed in terms of a stimulation index (SI) relative to cultures not containing drugs or the corresponding metabolites. Lymphocytes were incubated in the presence of (a) the indicated concentrations of sulfadoxine (circles) or pyrimethamine (squares), and (b) serum obtained from a healthy donor who had ingested either sulfadoxine (circles) or pyrimethamine (squares) at the indicated times before the serum was obtained. The patient's lymphocytes were stimulated by sulfadoxine but not by pyrimethamine, whereas the lymphocytes from the control subject responded to none of the additions ($SI = 2.5$ for patient's lymphocytes incubated in the presence of $10 \mu\text{g/ml}$ sulfadoxine and 3.4 for patient's lymphocytes incubated in serum obtained four hours after ingestion of sulfadoxine; $p < 0.001$ for comparison of the responses of the patient's lymphocytes with those of the control subject).

after steroid treatment. Further evidence for Fansidar as the pathogenic agent comes from the positive result in the lymphocyte transformation test, indicating sensitisation of the patient's lymphocytes to sulfadoxine (and not pyrimethamine). Thus a hypersensitivity reaction to sulfadoxine seems to be the most likely cause for pneumonia in this case.

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