Neutropenia during treatment of rheumatoid arthritis with levamisole

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SUMMARY   Of 60 patients with rheumatoid arthritis treated with levamisole 35% showed a persistent decrease in neutrophil counts from pre-treatment levels and 6 (10%) developed severe neutropenia of less than $1 \cdot 0 \times 10^9/1$. One of these neutropenic patients recovered without stopping therapy and the other 5 patients recovered rapidly when the drug was withdrawn. In some patients neutropenia recurred on reinstitution of levamisole.

Levamisole (L-1-(1) 2, 3, 5, 6-tetrahydro-6-phenylimidazo (2, 1-b) thiazole) is an immunoregulatory drug undergoing trials in a number of chronic inflammatory and neoplastic conditions (*Lancet*, 1975), which has been shown to be effective in the treatment of rheumatoid arthritis (Huskisson *et al.*, 1976; Rosenthal *et al.*, 1976a). A number of case reports of agranulocytosis, sometimes of life-threatening severity, in patients receiving levamisole have appeared in the literature (Rosenthal *et al.*, 1976b; Ruuskanen *et al.*, 1976; Sany *et al.*, 1976; Williams, 1976; Clara and Germanes, 1977; Van Holder and Van Hove, 1977). The present paper reports the results of haematological supervision of 60 patients with rheumatoid arthritis treated for 6–24 months with levamisole. Neutropenia occurred in a proportion of patients and its incidence, duration, and severity are documented.

Methods

Sixty patients with classical or definite rheumatoid arthritis as diagnosed by the American Rheumatism Association criteria (Ropes *et al.*, 1959) were treated for 6–24 months with levamisole in addition to their usual anti-inflammatory or analgesic drug regimens. Two dosage schedules were used. Forty-eight patients were treated with 50 mg of levamisole daily, increasing to a maintenance dose of 50 mg 3 times daily during the first month (continuous therapy), and 12 patients were treated with 50 mg 3 times daily for 3 consecutive days in each week (intermittent therapy). A placebo group of 12

patients with rheumatoid arthritis were treated with a red tablet containing lactose 3 times daily. None of the patients was known to have suffered previously from neutropenia or thrombocytopenia nor gave a history suggestive of such.

Disease activity was assessed by methods previously described (Huskisson *et al.*, 1976) including measurement of pain relief by a visual analogue scale (Scott and Huskisson, 1976). Haematological indices including haemoglobin, erythrocyte sedimentation rate (ESR), total and differential white cell count, and platelet count were measured before starting levamisole and then at monthly intervals.

All the patients were advised to contact their physician immediately if they became sick, and particularly if they developed mouth ulcers or febrile illnesses. When severe neutropenia (absolute neutrophil count $1 \cdot 0 \times 10^9/1$) developed, patients underwent prompt sternal bone marrow examination including Colony Forming Unit assay by a diffusion chamber technique (Gordon *et al.*, 1975).

STATISTICAL METHODS
Haematological and clinical changes were correlated using the Spearman's rank correlation test.

Results

NEUTROPHIL COUNTS
The pre-treatment neutrophil counts of all patients were within the normal range of $2 \cdot 0 – 7 \cdot 5 \times 10^9/1$, and striking changes occurred with levamisole therapy. Twenty-one patients (35%) showed a decrease of 30% or more from pre-treatment levels for at least 3 consecutive months, and 11 of these patients (18%) developed neutropenia, defined as an absolute neutrophil count below $2 \cdot 0 \times 10^9/1$ (Dacie

and Lewis, 1975). Six patients developed severe neutropenia with neutrophil counts of less than $1\cdot0 \times 10^9/1$; details of these patients are given in Tables 1 and 2. Levamisole therapy was withdrawn in 5 of these 6 patients but was not interrupted in any of the other patients, whose neutrophil counts returned to pre-treatment levels in 3–6 months.

The development of severe neutropenia occurred after a variable duration of levamisole therapy (1–23 months). It occurred in patients receiving both continuous and intermittent therapy, and in 4 patients there was mild neutropenia ($1\cdot4$–$2\cdot0 \times 10^9/1$) in the previous month.

None of these patients with severe neutropenia complained of symptoms suggestive of agranulocytosis and all the episodes were discovered on routine monthly blood counts. Nevertheless, on specific questioning, 5 patients did admit to having a sore mouth or mouth ulcers for a few days. One patient (case 2) became febrile on hospitalisation and although, on careful bacteriological investigation, no focus of infection was found, this rapidly resolved with antibiotic therapy. This patient was concurrently receiving long-term prednisolone (10 mg daily).

Neutropenia was not accompanied by any reduction in haemoglobin but in one patient (case 5) the platelet count fell to $88 \times 10^9/1$ ($88\ 000/mm^3$) and recovered to normal within 2 days. Sternal bone marrow examination at presentation was performed in 5 patients (Table 2). In 2 the distribution

and numbers of marrow elements were apparently normal while in the other 3 there was a striking reduction in granulopoiesis with normal numbers of myeloblasts and promyelocytes but a marked reduction of cells of the granulocyte series beyond this stage. Corresponding reductions in the colony forming ability of the bone marrow cells in culture were found in the latter 3 patients—the formation of 'large clusters' of 20–40 cells rather than 'colonies' of more than 50 cells is probably evidence of abnormal granulopoiesis.

In 5 patients with severe neutropenia (cases 2–6) withdrawal of levamisole therapy was followed by rapid recovery of the peripheral neutrophil count within 10 days. Repeat sternal bone marrow examinations in those patients with initial granulopoietic depression (cases 2–4) showed recovery of the marrow granulocyte precursors to normal numbers and distribution. In one patient the colony forming ability of the marrow cells in culture was reassessed and was also found to have returned to normal ($62\cdot8$ colonies/2×10^5 cells).

One of the patients with severe neutropenia (case 1), who had a normal bone marrow showed evidence of recovery of the circulating neutrophil count within 24 hours, before levamisole therapy was withdrawn, and treatment was therefore continued with complete recovery after 6 days. Intermittent levamisole therapy was reintroduced in 4 other patients (cases 3–6). Severe neutropenia recurred in 3 of these after a period of 1 to 9 weeks, requiring

Table 1 *Duration of severe neutropenia and relationship to levamisole therapy*

Case	Age (years)	Sex	Levamisole regimen	Duration of therapy before neutropenia (months)	Duration of neutropenia (days)
1	65	M	Intermittent	3	6
2	55	F	Continuous	1	10
3	65	M	Intermittent	13	5
4	53	F	Continuous	12	7
5	44	F	Continuous	19	1
6	39	F	Continuous	23	1

Table 2 *Haematological investigations*

Case	Pre-treatment neutrophil count ($\times 10^9/1$)	Neutrophil count 1 month before severe neutropenia ($\times 10^9/1$)	Lowest neutrophil count ($\times 10^9/1$)	Bone marrow appearance at lowest neutrophil count	Colony-forming units on in vitro marrow culture ($\times 2 \times 10^5$ cell*)
1	2·79	2·36	0·50	Normal granulopoiesis	74 colonies
2	5·11	5·11	<0·10	Promyelocyte 'arrest'	0
3	6·08	1·74	0·24	Promyelocyte 'arrest'	52 large cluster
4	6·56	1·34	0·34	Promyelocyte 'arrest'	26 large cluster
5	2·60	1·86	0·90	Not examined	Not examined
6	5·01	2·00	0·95	Normal granulopoiesis	70·5 colonies

*Normal: 60–100 colonies/2×10^5 cells; no large clusters.
Conversion: SI to traditional units—Neutrophil count: $1 \times 10^9/1 = 1 \times 10^3/mm^3$.

drug withdrawal. The fourth patient (case 5), while maintaining adequate neutrophil counts on intermittent therapy, developed recurrent severe neutropenia within 7 days of being transferred to continous therapy and levamisole was stopped with prompt recovery. Reintroduction of levamisole in intermittent therapy has not been accompanied by further severe neutropenia.

OTHER EFFECTS

The 60 patients treated with levamisole showed a slow improvement in disease activity as described previously (Huskisson *et al.*, 1976), accompanied by a rise in haemoglobin and a fall in ESR. Many patients showed a small decrease in the platelet count, but in only one did this fall below 150 $\times$ $10^9/l$ (see above). All patients had pre-treatment lymphocyte counts within the normal range of $1 \cdot 5$–$4 \cdot 0 \times 10^9/l$ (Dacie and Lewis, 1975). Changes in lymphocyte counts with levamisole therapy were variable: 17 patients (28%) showed a decrease of 30% or more from pre-treatment counts for at least 3 consecutive months while 6 patients (10%) showed an increase of similar magnitude.

There were no difference in changes in lymphocyte or neutrophil counts between patients receiving continuous or intermittent therapy (P >0·1) and no correlation with pain relief was found (r = 0·21) for neutrophils; for lymphocytes r = 0·3, P>0·1.

The patients receiving placebo therapy developed no significant haematological abnormalities during the study period.

Discussion

The data presented show that levamisole may have a marked depressant effect on the circulating neutrophil count of some patients with rheumatoid arthritis. In this group of 60 patients, 6 (10%) developed severe neutropenia and a further 15 (25%) showed a persistent decrease in neutrophil count from pre-treatment levels for period of 3 months or more. Neutropenia can occur at any time during treatment, is independent of whether therapy is given in a continuous or intermittent regimen, and is usually gradual in onset, although it can occur suddenly. In the cases described here prompt recovery followed withdrawal of the drug, and in 1 case recovery occurred despite continued therapy.

Nevertheless, neutropenia is obviously a potentially serious side effect of levamisole therapy of rheumatoid disease and deaths have been reported (Ruuskanen *et al.*, 1976, Clara and Germanes, 1977). However, it may only occur in patients with rheumatoid arthritis, and was not reported in studies of patients with breast cancer (Rojas *et al.*, 1976) or recurrent aphthous ulceration (Lehner *et al.*,

1976). The fatal cases of agranulocytosis with levamisole occurred in patients who were concurrently receiving steroid therapy, and avoidance of steroids or other myelosuppressive drugs would seem advisable in patients receiving levamisole. Other precautions should include regular monthly blood counts with more frequent supervision if the neutrophil count falls below 50% of the pre-treatment level or to below $2 \cdot 0 \times 10^9/l$. All patients must be instructed to report to their physician immediately if they become sick, and particularly if they develop mouth ulcers, sore throats, or febrile illnesses. Treatment of neutropenia should be conservative, with immediate withdrawal of the drug and antibiotic therapy if there is evidence of infection until the neutrophil count recovers. Rapid recovery is usual and levamisole therapy can sometimes be reintroduced under close supervision.

The mechanism by which levamisole produces neutropenia is unknown. In some cases (Rosenthal *et al.*, 1976b; Van Holder and Van Hove, 1977) leucocyte aggulutinins have been detected in the serum of patients with neutropenia suggesting an immune-destructive mechanism. Clara and Germanes (1977) postulate that levamisole could act as a hapten in a hypersensitivity reaction. However, the tolerance of some of the patients in this study to reintroduction of the drug would tend to argue against a hapten theory in every case as does the spontaneous recovery of the neutrophil count in one patient in whom the drug was not withdrawn.

Only 3 of the patients with severe neutropenia showed evidence of marrow depression of granulopoiesis with a marked reduction of granulocyte precursors beyond the promyelocyte stage and a marked impairment of *in vitro* colony forming ability. In 2 other patients neutropenia co-existed with apparently normal marrow appearances and a normal capacity to form granulocyte colonies in culture. These observations may suggest that levamisole causes neutropenia by two different mechanisms—a marrow toxic effect and a peripheral neutrophil destruction, which may possibly be immune-mediated. Since marrow appearances recovered rapidly, those patients with normal marrow appearances may have been in the recovery phase of a severe transient neutropenia when examined.

It has been suggested that some treatment of rheumatoid arthritis may act by a reduction of circulating lymphocytes (Hurd and Ziff, 1974). In this study no correlation was found between changes in lymphocyte or neutrophil counts and changes in pain relief. It would appear unlikely, therefore, that this is the mode of action of levamisole in rheumatoid arthritis.

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