Intra-articular methotrexate

Clinical and laboratory study in rheumatoid and psoriatic arthritis

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SUMMARY The effects of intra-articular methotrexate (MTX) were compared with saline in 20 patients with persistent knee effusions due to rheumatoid arthritis (15) and psoriasis (5) in a double-blind pilot study. Clinical improvement was seen in most patients given either MTX or saline and was attributed to joint irrigation during arthroscopy and to placebo effects.

MTX had a local anti-inflammatory effect in the psoriatic arthropathies; the percentage of polymorphonuclear cells and pyroninophilic mononuclear cells in synovial fluids fell sharply. Intra-articular hydrocortisone acetate was not anti-inflammatory in 2 psoriatic patients treated subsequently. The anti-inflammatory action of MTX in joints may resemble its effectiveness in controlling the rash of psoriasis.

Rheumatoid arthritis (RA) and associated arthropathies are chronic diseases with no known cure. Intra-articular (i.a.) injection of corticosteroids is often followed by transient relief of symptoms and reduction of local synovitis (Hollander, 1972), but not in all cases. A few patients with persistent knee effusions which did not respond to i.a. corticosteroids were given MTX i.a. and the effusions did not reappear for several months (Hall and Head, 1975). MTX, a folic acid antagonist, has been used successfully in controlling the rash of psoriasis (Weinstein, 1977) and has been used systemically to control the arthropathy of psoriasis (Sigler, 1972) but with severe side effects (Black *et al.*, 1964). We suggest that local application of MTX in small doses avoids the toxicity of oral or intravenous therapy.

This pilot study was set up to discover whether MTX is a suitable treatment for intra-articular use in both RA and psoriatic arthritis. Because assessment of clinical improvement is notoriously difficult in these arthropathies, where relapse and remission are common, we chose (i) to have a control group of patients given saline, although for ethical reasons the joints of both test and control groups were irrigated with saline, and (ii) to measure changes in the abnormalities of synovial fluid by laboratory tests, namely, total leucocyte counts, the % of

polymorphonuclear leucocytes (polymorphs) as a measure of inflammation, and IgG-class rheumatoid factor titre (IgG RF) to assess local immunological abnormalities. Intra-articular corticosteroids have been assessed by similar tests (Goetzel *et al.*, 1974). Large pyroninophilic mononuclear cells, which are found in large numbers in fluid from arthritic joints, were also studied as a possible indicator of successful therapy.

Materials and methods

PATIENT SELECTION

Twenty patients with persistent inflammation in one or both knees were selected for the trial. Patients either fulfilled the ARA criteria for RA (15) or had polyarthritis with psoriasis (5). Of the 15 RA patients 2 also had systemic lupus erythematosus and one had osteoarthritis; one of the psoriatics also had RA (Table 1). Five RA patients were receiving immunosuppressive therapy (azathioprine, cyclophosphamide, or prednisone) before or during the trial.

TREATMENT

The 20 patients were allocated treatment at random by raising 20 cards, 10 marked MTX and 10 saline; one card was drawn for each patient. With unilateral arthritis either MTX or saline was injected according to the card. With bilateral arthritis the material noted on the card referred to the right knee and the alternative was given to the left. The 20 patients fell

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Table 1 Summary of general and clinical data on patients

Group and treatment	No. of patients	Average age (yr) (range)	Males: females	Diagnosis (no. with associated diseases)	Average duration of disease (yr) (range)	Major concurrent drugs	No. with RF in serum	
							IgM RF	IgG RF
Group 1 Bilateral MTX/saline	10	52 (29-68)	3:2	8 RA; 2 psoriatic arthritis (1 psoriatic with RA; 2 RA with LE)	7.5 (4m-21)	2 on prednisone 1 on ACTH + cyclophosphamide	6	8
Group 2 Unilateral MTX	5	47 (22-71)	3:2	3 RA; 2 psoriatic arthritis	9.3 (1½-21)	1 on prednisone	3	3
Group 3 Unilateral saline	5	61 (55-65)	3:2	4 RA; 1 psoriatic arthritis (1 RA with OA)	9.2 (1-26)	1 on azathioprine	3	4

into three groups: group 1 (10 patients) had MTX one side and saline the other; group 2 (5 patients) had MTX unilaterally; and group 3 (5 patients) had saline unilaterally.

Intra-articular injections were given on days 0, 7, and 14. Each dose of MTX (Lederle Laboratories) was 10 mg except for the first 3 patients who received 2.5 mg doses. Saline doses were 5 ml. The trial was double-blind, neither the patient nor the assessor knowing the nature of the injection.

Arthroscopy was performed on day 0 and after 3 months using an Olympus fibroscope and the joints were washed out with saline just before injection. Appearances were classified as (i) quiescent, with no evidence of villous hypertrophy, inflammation, or exudate; (ii) active inflammation with red hypertrophic villi; and (iii) degenerative changes, fibrinous networks, and fibrinous exudates.

CLINICAL ASSESSMENT

Patients were assessed before injection on days 0, 7, and 14 and also at 3 and 12 weeks for subjective improvement in general condition, knee pain at rest, knee pain on walking, and for the duration of morning stiffness. Objective measurements were knee circumference, synovial effusion (fluid volume), heel-pubis distance, and walking time for 40 m. Each test was scored +1 (improvement), 0 (no change), or -1 (deterioration) since the previous visit and was not compared with the initial state.

LABORATORY TESTS

As much synovial fluid as possible was withdrawn on day 0 and at 1, 2, 3, and 12 weeks. Total leucocytes/ml were recorded. Smears of fluid were stained with haematoxylin and eosin for % polymorphs and with methyl green-pyronin for the % of mononuclear cells which were pyroninophilic. Pyronin stains cytoplasmic ribosomal RNA and cells were classed as

positive if their cytoplasm to nuclear ratio was 25-50% and the cytoplasm stained intense red with pyronin.

Fluid and serum samples were assayed for IgG RF (anti-rabbit globulin) by radioassay (Hay *et al.*, 1975). Rheumatoid factor (IgM RF) (anti-human globulin) was determined by latex agglutination. Full blood counts and liver function tests were done before treatment and after 3 and 12 weeks.

SUBSEQUENT STUDIES

Subsequent to the trial the effectiveness of MTX compared with that of i.a. hydrocortisone acetate (Boots) in patients with psoriatic arthritis and RA. In addition to 10 patients from the trial, 3 other patients with persistent knee effusions, 2 with RA and 1 with psoriatic arthritis were studied. All patients included in this study are listed in Table and their treatment was the following.

Cases 1-4, 7, and 10-12 received MTX in the trial and Cases 5 and 8 saline, so all these patients also had had joint irrigation. When treated with MTX Cases 5 and 6 had 3 doses at weekly intervals, Case 7 had 5 mg into each knee and Case 6 had 10 mg into one knee; 2 patients (Cases 9 and 13) had single doses of 10 mg MTX into the treated knees. Each patient given hydrocortisone acetate had a single dose of 50 mg into the treated knee.

Results

GROUP COMPARABILITY

General and clinical data on the patients in the trial are summarised in Table 1. The average age, the ratio of males to females, the number of patients with RA compared to those with psoriatic arthritis, the average duration of disease, and the proportion of those with IgM RF and IgG RF are fairly evenly distributed between the three groups. It is unfortunate that 5 patients had to be maintained on

immunosuppressive drugs during the trial. They did not differ notably from the others, however, in clinical changes during the trial or in their pre- and post-treatment tests on synovial fluid. At arthroscopy, 26 of the 30 joints included in the trial had active disease with villous hypertrophy and inflammation. The remaining 4 showed degenerative changes.

CLINICAL ASSESSMENT

The results of clinical assessments were obtained by summing the scores for each patient in each test and averaging the values for each group. The total possible improvement in each test for each patient after 1, 2, 3, and 12 weeks was 1, 2, 3, and 4 points respectively, i.e. +1 for each assessment. For each test, the means per group are given as a percentage of the maximum possible improvement at 3 and at 12 weeks (Fig. 1). Similar but even smaller differences between the groups were found at 1 and 2 weeks.

For the sum of all 8 clinical tests, the means per group are given as a percentage of maximum possible improvement at 1, 2, 3, and 12 weeks (Fig. 2).

MTX had no clear beneficial effect over saline on any clinical component. Treatment of both knees could explain the considerable improvement in walking time in group 1 compared with the unilateral treated groups. Overall improvement was seen in all three groups at all four assessment times and the best improvement was seen in the first 3 weeks. Only one patient showed overall deterioration in symptoms (bilateral treatment) and one patient (saline) went into remission.

Joint irrigation at arthroscopy may have been a major factor. At the second arthroscopy at 12 weeks there appeared to be a reduction in fibrin content, less synovial inflammation, and fewer villi in about half the joints irrespective of whether they had been injected with MTX or saline. Removing most of the fluid each time may also have been beneficial. These two factors could account for the fall in fluid volume in all three groups after 3 weeks and for the gradual fall in leucocyte count and % polymorphs in all groups over the 12-week period (Table 2).

LABORATORY TESTS

Table 2 gives the mean values for each group for the volume of synovial fluid taken at day 0, 3, and 12 weeks including those knee joints with no fluid; the number of joints having fluid at these times is also noted. The mean values for total leucocyte count per ml, % polymorphs, the % of mononuclear cells which were pyroninophilic, and the levels of IgG RF include only values from joints with fluid.

Fig. 1 Comparison of clinical improvement in each test at 3 and at 12 weeks. ■ Group 1, bilateral MTX and saline; ▨ group 2, unilateral MTX; □ group 3, unilateral saline. For each test the mean of the scores up to 3 weeks and up to 12 weeks is given as a percentage of the mean maximum possible improvement at 3 and 12 weeks (3 and 4 points, respectively).

Fig. 2 Comparison of clinical improvement at each assessment time for the sum of all 8 tests. Groups as in Fig. 1. The means for the sum of all 8 tests at each assessment is given as a percentage of the mean maximum possible improvement in the period from the previous assessment (1 point).

The single clear-cut effect of MTX was to reduce the % of pyroninophilic cells in joint fluids. The MTX-treated patients (groups 1 and 2) had fewer pyroninophilic cells after 3 and 12 weeks; the saline control group had more. There was no marked change in any group in the mean levels of IgG RF. Only 2 of the psoriatic patients (Cases 3 and 4, Table 3) had levels of IgG RF above normal in their synovial fluid and one of these had associated RA.

In the bilaterally-treated patients MTX acted on the pyroninophilic cells in both knees although only one knee was injected. To follow the release of MTX from the synovial space, blood was taken from 2 patients $\frac{1}{2}$, 1, and 2 hours after the first MTX injection. MTX was detected in the circulation after

$\frac{1}{2}$ hour and had reached the maximum level of 140 to 160 ng/ml at 1 hour. Therefore it probably entered the contralateral knee space shortly after each injection.

COMPARISON OF PSORIATIC ARTHRITIS AND RA
The considerable drop in mean % polymorphs in group 2 patients after 3 weeks was contributed by 2 psoriatic patients (Table 2). Table 3 compares the effect of i.a. MTX in patients with psoriasis and in those with RA. The efficacy of MTX was also compared with that of hydrocortisone acetate in some patients treated with both drugs on different occasions. An interval of at least 2 months was allowed between treatments in patients treated

Table 2 Comparison of changes in synovial fluids after MTX or saline

Group and treatment	Fluid vol* (ml) (no. joints with fluid)			No. leucocytes† ($\times 10^6$ /ml)			% polymorphs†			Pyroninophilic† (% of mononuclear cells)			IgG RF† (ng anti-IgG bound)		
	0	3w	12w	0	3w	12w	0	3w	12w	0	3w	12w	0	3w	12w
Group 1															
Bilateral	16	4	14	15	10	6	77	84	62	48	50	20	88	97	93
MTX	(8)	(3)	(8)												
Saline	19	7	10	14	11	6	68	56	54	54	51	14	101	87	100
	(8)	(5)	(7)												
Group 2															
Unilateral	32	20	11	4	4	2	53	16	47	25	6	16	53	40	37
MTX	(5)	(3)	(3)												
Group 3															
Unilateral	27	16	19	8	9	3	76	67	61	8	26	11	65	32	44
Saline	(5)	(3)	(3)												

* Average for all joints in the group, including those with no fluid.
† Average of values from those joints with fluid.

Table 3 Comparison of changes in synovial fluid of psoriatic and RA patients given drugs or saline

Case no.	Diagnosis	RF in serum		MTX				Hydrocortisone				Saline			
		IgM	IgG	% polymorphs		% pyroninophilia		% polymorphs		% pyroninophilia		% polymorphs		% pyroninophilia	
				Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	Psoriasis	—	—	74*	11	39	6	ND		ND		ND		ND	
2	"	—	—	48*	7	19	5	ND		ND		ND		ND	
3	" + RA	—	+	72*	8	20	12	8	67	1	29	ND		ND	
4	"	—	—	72*	NF	60	NF	ND		ND		ND		ND	
5															
L knee	"	—	—	73	37	17	4	64	60	10	8	74*	67	8	18
R knee	"	—	—	69	31	9	5	81	57	8	5	69*	57	13	15
6	"	—	—	75	41	10	<1	80	70	11	20	ND		ND	
Average				69	24	25	6	58	65	8	16				
7	RA	—	+	77*	72	96	57	52	52	22	9	ND		ND	
8	"	+	+	ND		ND		70	3	8	>1	80*	77	<1	48
9															
L knee	"	—	+	82	87	23	18	87	86	18	56	ND		ND	
R knee	"	—	+	82	91	30	8	91	90	8	15	ND		ND	
10	"	+	+	5*	18	17	6	ND		ND		ND		ND	
11	"	+	+	82*	92	72	63	ND		ND		ND		ND	
12	"	+	+	87*	87	15	31	ND		ND		ND		ND	
13	"	+	ND	87	81	45	<1	81	40	<1	4	ND		ND	
Average				72	75	35	26	76	54	11	17				

* Arthroscopy and irrigation.
ND = not done; NF = no fluid.

consecutively. All patients had MTX before hydrocortisone but Cases 5 and 8 had saline and joint irrigation before the drugs.

The % polymorphs and % of mononuclear cells which were pyroninophilic in joint fluids are given for each patient before treatment and for a sample taken from 2 to 6 weeks later (Table 3). All psoriatic patients responded to MTX with a fall in % polymorphs and % pyroninophilic cells, except Case 4 who had no fluid after MTX, but the response to subsequent treatment with hydrocortisone was negligible. In the RA patients, however, MTX did not reduce fluid polymorphs although in most patients the % of pyroninophilic cells was reduced. Hydrocortisone, as expected, was anti-inflammatory in the RA patients but only in the seropositive patients and not in the 2 seronegative patients.

Discussion

As measured by laboratory tests intra-articular MTX had a short-term anti-inflammatory effect on patients with psoriatic arthritis but not on those with RA. This anti-inflammatory effect could not be correlated with clinical improvement because clinical symptoms improved in all but one patient anyway. Our results underline the importance of including a control group of patients not given drugs and of using both laboratory and clinical tests before drawing conclusions.

This assessment of i.a. MTX follows our preliminary report suggesting that MTX benefits various arthropathies with persistent effusions (Hall and Head, 1975). Marks *et al.* (1976) were unable to confirm this report probably for the following reasons. They treated seropositive RA patients but not patients with psoriatic arthritis; they used steroids with MTX in the same patient, and steroids can inhibit certain activities of MTX (Chabner *et al.*, 1975); the assessment was done on clinical components alone. Intra-articular MTX has slight anti-inflammatory effect in patients with RA detected by thermography (Bird *et al.*, 1978) but this present study indicates that MTX is strongly anti-inflammatory only in certain arthropathies, mainly the psoriatics, as judged by laboratory tests. Clinical assessment did not show this because all patients improved including those given saline, particularly during the first 4 weeks after arthroscopy and after removal of as much fluid as possible each week. Joint irrigation is known to be temporarily beneficial and was done to the control patients for ethical reasons. Earlier reports (Black *et al.*, 1964; Sigler, 1972) that systemic MTX benefits the arthropathy in psoriatic patients support our conclusions. MTX given intra-articularly is apparently safer and less toxic as none of our patients or those of Marks

et al. (1976) treated by this route developed abnormal liver function tests or blood tests.

The most commonly used clinical indicator of intra-articular drug effectiveness is abatement of effusions; this is how the benefits of i.a. MTX were judged initially (Hall and Head, 1975). In our trial, however, after fluid was withdrawn it reappeared at such widely different times and in differing volumes that it was not possible to assess the role of MTX on this basis. However 5 of the psoriatic patients had no fluid for up to 12 weeks after i.a. MTX. Fluid in the sixth patient did not disappear after MTX but did so temporarily after hydrocortisone.

The fall in % polymorphs in synovial fluids from psoriatic patients was the best indicator of an anti-inflammatory effect of MTX. It resembles the fall seen in RA patients after i.a. corticosteroids (Goetzel *et al.*, 1974). All but one of the patients treated with corticosteroids by Goetzel *et al.* also improved clinically. Their control was a saline injection into the contralateral knee. But these control knees also improved, judging by decrease in local symptoms and in % polymorphs and this was attributed either to systemic effects of corticosteroids or to the process of aspiration and injection alone. Similar changes in synovial fluid polymorphs and in pyroninophilic cells were found in both the saline- and MTX-treated knees of our bilaterally-treated patients. We can attribute the change seen in the saline-treated knees of these patients to MTX for two reasons: because MTX injected into the knee space appeared rapidly in the circulation and because no fall in % pyroninophilic cells occurred in patients given saline unilaterally. We conclude therefore that the contralateral knee is not a useful control for intra-articular drugs.

Large pyroninophilic mononuclear cells appear in fluid in actively arthritic joints whether psoriatic or rheumatoid. The percentage of these cells and intensity of staining was reduced in most cases by MTX and less often by hydrocortisone. Pyroninophilic cells are involved in cell division and in protein synthesis. Large pyroninophilic cells appear in lymphatic tissues shortly after antigenic stimulation as the precursors of immunologically committed small lymphocytes (Turk and Stone, 1963). Intensely pyronin-positive cells in the normal synovial lining synthesise various proteins (Williamson *et al.*, 1966). Many of those in the rheumatoid synovium are plasma cells synthesising immunoglobulin (Johansen, 1965; Ziff, 1974). These 'lining' cells and plasma cells are only occasionally seen in synovial fluids. The majority of large pyroninophilic cells commonly found in fluid from psoriatic and rheumatoid joints are probably monocytes, not lymphoblasts, because many adhere to glass and have intense diffuse non-specific acid alpha-naphthyl-acetate esterase activity

(unpublished observations). In psoriatic arthritis they may not have the same role as in the postulated immune complex-induced inflammation of RA. Nevertheless, these cells are more metabolically active than those in blood and a reduction in their numbers might help to eradicate the disease process whatever the underlying mechanisms.

MTX acts on cells synthesising RNA, DNA, and protein (White *et al.*, 1975) and would be expected to affect these pyroninophilic cells. *In vivo*, MTX fails to prevent the appearance of large pyroninophilic cells but does prevent their subsequent division into daughter cells (Turk and Stone, 1963). The % pyroninophilic cells in joint fluids rose in the first few days immediately after i.a. MTX in 3 RA patients studied daily but subsequently fell to below pretreatment levels (unpublished observations). In the first few days perhaps cells already in cycle were arrested in their synthetic phase by MTX and more cells came into cycle. Then later, as MTX inhibited the development of daughter cells, fewer cells were available to enter the cell cycle and become pyroninophilic. We conclude that measurement of these pyroninophilic cells is a useful indicator of drug activity, particularly for cytotoxic antimetabolites like MTX (Bertino, 1973).

From a clinical viewpoint this study has indicated that MTX is not a toxic treatment given intra-articularly in small intermittent doses and that psoriatic arthropathies may benefit more from MTX than from hydrocortisone. Clinicians using intra-articular cytotoxic drugs may find that, apart from the patient's subjective opinion, the best objective short-term assessment would be serial investigations of synovial fluid by routine counting of the percentage of polymorphs to estimate the anti-inflammatory effect of the drug and of % pyroninophilic cells to estimate its cytotoxic activity.

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