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Rheumatoid knee synovitis successfully treated with intra-articular rifamycin SV

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SUMMARY Thirty rheumatoid patients with persistent knee effusion were treated intra-articularly with rifamycin SV, 500 mg weekly, or with saline solution, 10 ml, in a double-blind study. A complete disappearance of effusion and an impressive clinical improvement was observed in the patients on rifamycin. The synovial fluid and membrane underwent some changes. In 2 patients the rifamycin caused a painful local reaction. After a follow-up of 5 years only one patient has experienced effusion relapse, 5 months after the termination of rifamycin SV treatment. The patients on saline showed no significant change. On the basis of the results obtained from the monoarthritis experimental model and from clinical trials it is tempting to consider that rifamycin has an antimitotic effect, impeding the synthesis of RNA and DNA polymerases in immunocompetent cells.

Compounds with inhibitory activity on cell growth such as alkylating agents and antimetabolites have been extensively used in the treatment of rheumatoid arthritis (RA) because of their potential anti-immune and anti-inflammatory properties. The results of clinical trials have been uncertain,[1,2] while the incidence and severity of side effects have severely limited their usefulness.[3]

The intra-articular use of cytotoxic drugs has been limited because of local and general toxicity and has met with little success.[4–10] Early experiments demonstrated that rifamycin SV, a valuable antibiotic with a wide spectrum of antibacterial properties, inhibited the protein synthesis of bacterial cells by blocking DNA-dependent RNA polymerase.[11] We decided on the use of rifamycin SV in RA after observing that, when injected locally in experimental monoarthritic guinea-pigs, it markedly inhibited the uptake of labelled precursors of RNA and DNA by inflammatory cells and hyperplastic lining cells, with no histological evidence of cellular necrosis (Caruso I, unpublished).

The data presented here are the results of local treatment of RA knee synovitis in a double-blind study in which rifamycin SV was compared with saline solution.[12,13] From these results we can conclude that rifamycin SV is a promising development in the field of local RA therapy.

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Materials and methods

Thirty patients with persistent knee effusion and a similar activity grade of local disease (see clinical assessment) were chosen for intra-articular treatment. All of them met the American Rheumatism Association criteria for RA. Patients were excluded if they had taken gold salt, immunosuppressive agents, levamisole, or penicillamine in the past 6 months, or had raised blood urea nitrogen (BUN) or serum creatinine, or total bilirubin or alkaline phosphatase. The patients were placed at random in the rifamycin and saline groups. Both groups were treated intra-articularly every 7 days, the former with 500 mg of rifamycin SV (10 ml of arginine salt of rifamycin SV in bidistilled water: Lepetit Spa, Milan), and the latter with 10 ml of saline solution. Synovial fluids were completely drained off before each infiltration. On the basis of our preliminary experience the duration of treatment was planned for 12 weeks, but it was withdrawn whenever the effusion disappeared. All patients were examined every 3 months for 5 years. The presence of effusions and clinical signs of local inflammation were assessed.

Although there was a difference in colour between the rifamycin and saline solutions, the blind schedule was achieved with the aid of a colleague from the medicinal department who injected the rifamycin SV or saline solution in random order and with no supervision from us. He did not participate in any other

part of this study. Neither the rheumatologist who performed the clinical assessment nor the patient knew which medicine had been administered.

CLINICAL ASSESSMENT

General condition and local abnormalities were assessed on an arbitrary scale before each knee aspiration.[10 14 15] The subjective parameters of pain at rest, pain on movement, and duration of morning stiffness were all graded from 0 to 3. Objective measurements were graded as follows: knee swelling due to fluid and/or soft tissue involvement 0–3; synovial fluid volume 0–3; radiological damage 0–1; ballottement of patella 0–1; general condition 0–1. An *individual score* was obtained by adding up scores of all the subjective and objective tests to a maximum of 18 points. This score was divided into 3 grades: 0–5, mild; 6–11, moderate; over 12, severe. The *total score* for each test was obtained by adding up the scores of all the patients and was expressed as a percentage of the maximum possible score. Since the number of infiltrations needed for the disappearance of effusions was not the same in all patients, 4 points were chosen to indicate more clearly the time course of treatment: basal score, midpoint (i.e., half the number of infiltrations), end of treatment, and 12 weeks after treatment.

LABORATORY TESTS ON BLOOD

Laboratory tests were performed before the beginning of treatment, after 4 weeks, and at the end of the trial, and covered haemoglobin, total and differential white blood cell count, platelet count, ESR, BUN, creatinine, total bilirubin, serum transaminase (SGOT), serum alkaline phosphatase, and urine analysis.

LABORATORY TESTS ON SYNOVIAL FLUID

The synovial fluid (SF), as much as possible being aspirated before each injection, was immediately analysed for total and differential leucocyte count and RA cells.

Biochemical tests included: glucose (autoanalysis); protein[16]; β-glucuronidase (Talalay *et al.*[17] modified by Kerr and Leuvy[18]) with phenolphthalein glucuronide as the substrate (Sigma Chemical Co); acid phosphatase[19] with nitrophenylphosphate as the substrate (Boehringer Mannheim GmbH).

Immunological studies included C3–C4 complement fractions determined by the radial immunodiffusion method.[20] T lymphocytes were detected by sheep red blood cells rosette (E) formation following Jondal *et al.*[21]; B lymphocytes were identified by the presence of surface immunoglobulins following Aisemberg and Bloch[22]; A, G, M immunoglobulins were tested for with commercial immunoplates.

The rheumatoid factor was tested for by the latex fixation test and by sensitised sheep cell agglutination.[23] Prostaglandins E_2 and $F_{2\alpha}$ were assayed by RIA; 150 μl aliquot parts of the unextracted samples were analysed in triplicate at a 1:10 dilution in the standard diluent of the assay, as previously described.[24] The details of the binding affinity and the immunological specificity of anti-PGE_2 and anti-$PGF_{2\alpha}$ have been described elsewhere.[25] With 6000–8000 dpm of ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$ (Amersham Radiochemical Centre: 120–170 Ci/mM) in a volume of 1·5 ml, the lowest detectable concentration of both compounds was 20 pg/ml of SF.

HISTOLOGY OF SYNOVIAL MEMBRANE

Needle biopsies of the synovial membrane (SM) were performed before and 3 months after the end of treatment in 3 patients from both groups. Another 4 patients from the rifamycin group had a second biopsy after periods varying from 4 to 28 months. The tissue was fixed in buffered formaldehyde, and 5 μm thick paraffin sections were stained with haematoxylin-eosin. The synovial pattern was assessed without knowledge of the kind of intra-articular treatment or the time sequences of the biopsies. The degree of lymphoplasmacellular infiltration, of connective tissue extension, and hyperplasia of the synovial lining cell were evaluated on a graduated scale.[26]

Statistical analysis. The means and the standard deviations were calculated. The differences between mean values were determined by the *t* test.

Results

CLINICAL RESPONSE

In Fig. 1 the results of intra-articular therapy with rifamycin and saline solution are shown. Between the first infiltration and midpoint, which means half the total number of infiltrations needed for the disappearance of effusions, the clinical response was slight. At midpoint the synovial fluid volume decreased from 75% (basal value) to 50% in the rifamycin group; however, pain and morning stiffness did not change significantly. Between midpoint and the final infiltration all clinical parameters progressively improved until effusions completely disappeared in all rifamycin-treated patients together with a marked reduction of pain and morning stiffness. The number of infiltrations necessary to obtain the disappearance of synovial effusions ranged from 3 to 9. Although the synovial fluid was absent at the end of treatment, a certain degree of swelling, located mainly at suprapatellar bursae and due to soft-tissue involvement, was still apparent in many patients. Six weeks later the residual swelling disappeared too, and clinical

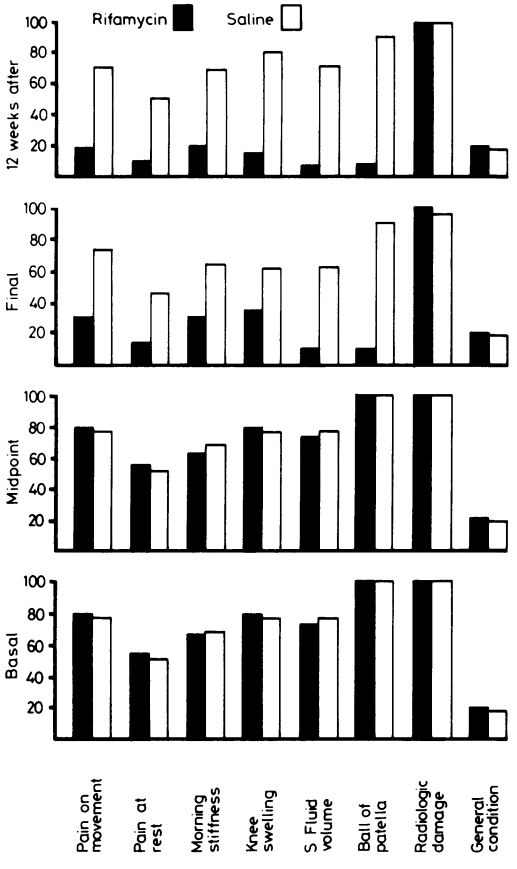

Fig. 1 *Clinical response to intra-articular rifamycin SV (500 mg weekly) and saline solution (10 ml) in 30 patients. For the various times shown the score for each test, which was obtained by adding up scores of all patients, is expressed as a percentage of maximum possible score.*

signs of synovitis were further improved. Systemic illness and clinical inflammation signs in the other joints were not modified. However, 2 patients with prevalent localisation in knee joints discontinued corticosteroid therapy.

Saline-injected patients showed a slight decrease of pain and synovial fluid volume after 12 infiltrations. These results did not change during the following 12 weeks (Fig. 1). One patient from the rifamycin group had a relapse of effusion 5 months after the end of treatment (Table 1), the remaining 14 patients have not so far had relapses for 5 years. They have not been treated with gold, antimalarials, penicillamine, or levamisole. Of the patients in the saline group one was lost to follow-up and 14 showed persistent effusions (Table 1), although the extent of these was generally in relation to fluctuation of systemic disease.

SYNOVIAL FLUID CHANGES

Table 2 shows the results of laboratory tests on the synovial fluid in the rifamycin and saline groups. The pretreatment and final values are given for each test. The latter refer to the synovial fluid aspirated at the final infiltration. At this point the reduction of SF volume reached 81% in the rifamycin group and 22% in the saline one. In both groups all parameters showed a wide range of variation during the course of treatment. A comparison between the basal and final values in the rifamycin group showed a significant reduction in leucocytes and the percentage of polymorphonuclear leucocytes ($p<0{\cdot}01$). These changes were not consistent in all patients; leucocytes decreased in 12 and increased in 3. Polymorphonuclear leucocytes decreased in 11 and increased in 4. On the other hand leucocytes in the saline group increased by 16% and polymorphonuclear leucocytes by 12%, but the comparison with the basal value was not statistically significant. The rheumatoid arthritis cells too showed a contrary tendency in the 2 groups, being decreased by 16% in the rifamycin group and increased by 20% in the saline group. Protein, glucose, C3 and C4 complement fractions, β-glucuronidase, acid phosphatase, immunoglobulins, RF titre, and the number of T and B lymphocytes were substantially unchanged in both groups. A comparison of basal and final concentrations of prostaglandins did not show any statistically significant change. However PGE_2 were reduced by 51% and $PGF_{2\alpha}$ by 15%.

HISTOLOGY

The synovial membranes of rifamycin-treated patients showed a reduction in synoviocytes and fibroblasts in the superficial layers and diffuse infiltrates in subsynovial tissue. In many samples a larger amount of fibrin than usual was deposited in clumps, which underwent organisation and assumed a connective tissue structure; some masses of fibrin remained as inert material, being delimited by palisading synoviocytes. These changes were present in widely varying degrees both in different patients and in the same synovial sample. The histological features of SM in the rifamycin-treated patients did not correlate either with the number of infiltrations or with the period of time after treatment.

Table 1 *Follow-up of RA knee synovitis*

Months from the end of treatment	*Rifamycin SV*	*Saline*
	Effusions/patients	
1—	0/15	15/15
3—	1/15	15/15
6—60	1/15	14*/14

*One patient lost to follow-up.

Table 2 *Changes in synovial fluid after rifamycin or saline*

	Rifamycin					*Saline*				
	No. patients	*No. serial determinat.*	*Basal mean±SD*	*Final mean±SD*	*Statistic. signific.*	*No. patients*	*No. serial determinat.*	*Basal mean±SD*	*Final mean±SD*	*Statistic. signific.*
Leucocytes (per mm³)	15	95	11725 ±6500	5574 ±3700	p<0·01	15	93	9580 ±5070	11380 ±5080	p NS
Polys (per cent)	15	95	65·89 ±14·48	41·15 ±15·72	p<0·01	15	93	52·26 ±25·49	59·26 ±28·01	p NS
RA cells (per cent)	15	90	28·62 ±20·87	24·20 ±17·34	p NS	15	90	22·66 ±11·87	28·40 ±13·85	p NS
Protein (g/100 ml)	15	93	3·93 ±0·91	4·01 ±0·90	p NS	14	90	3·92 ±0·75	4·03 ±1·03	p NS
IgG (mg/100 ml)	15	95	878 ±428	948 ±392	p NS	13	82	850 ±257	816 ±185	p NS
IgA (mg/100 ml)	15	95	152 ±91	140 ±81	p NS	13	80	127 ±77	139 ±93	p NS
IgM (mg/100 ml)	15	95	118 ±68	118 ±73	p NS	13	80	98 ±32	100 ±31	p NS
C3 (mcg/ml)	15	95	438 ±199	490 ±213	p NS	13	84	524 ±268	563 ±268	p NS
C4 (mcg/ml)	15	95	96 ±62	105 ±69	p NS	13	84	100 ±45	103 ±48	p NS
T lymphocytes (per cent)	15	92	56·16 ±18·86	57·41 ±14·16	p NS	15	60	57·07 ±16·08	57·23 ±19·52	p NS
B lymphocytes (per cent)	15	94	7·25 ±4·49	8·08 ±4·44	p NS	15	60	7·15 ±2·33	7·53 ±2·63	p NS
PGE_2 (pg/ml)	10	65	81±65	39±14	p NS					
$PGF_2\alpha$ (pg/ml)	10	65	33±17	28±16	p NS					

The RF titre did not change more than one tube in serial determination. At the aspiration before the last infiltration synovial fluid volume was reduced by 81% in the rifamycin group and by 22% in the saline group.

SIDE EFFECTS

In many patients the intra-articular injection of rifamycin SV was followed by local pain of variable intensity due to the irritative properties of the drug. The pain lasted some minutes. The immediate increase of local heat was appreciable too. Besides the initial, irritative pain the rifamycin SV caused a delayed local painful reaction in 2 of the 15 patients. This reaction took place between the third and fifth infiltrations and happened from 6 to 8 and sometimes from 12 to 14 hours after infiltration. The injected knee became warmer and the tension increased because of increased fluid; the temperature rose by some degrees and, after reaching rarely 39°C, fell again within 1–2 hours. The reaction subsided after some hours but sometimes continued all night. The outcome was not related either to intensity or to frequency of local reactions.

Discussion

The results of our trial clearly demonstrate that local treatment with rifamycin SV is of excellent therapeutic effect in rheumatoid knee synovitis (Fig. 1). All patients experienced a gradual and complete disappearance of knee effusions and most showed a notable improvement in local symptoms. In 2 patients with the disease mainly localised in the knee joints corticosteroid therapy was discontinued.

Histological examination at various intervals after treatment showed the persistence of synovitis, although a certain degree of cellular depletion and newly formed connective tissue were present. It is difficult to reconcile this finding with the low frequency of recurrence. In fact only one of the 15 patients had a relapse of effusion 5 months after the end of treatment. The remaining 14 patients, have not so far had relapses for 5 years. All the 15 patients of the saline group showed persistent effusions, although the extent of these was generally related to fluctuation in systemic disease (Table 1). Synovial fluid changes in RA patients suggest the hypothesis that rifamycin SV has a potent anti-inflammatory effect. The synovial fluid volume at the final infiltration was reduced by 81%, and correspondingly the total content of both cellular and soluble components was reduced by nearly the same amount. Leucocytes per mm³ and the percentage of polymorphonuclear leucocytes further decreased so that the comparison between basal and final values yielded significant differences ($p<0·01$). The reduction in both the relative and absolute content (Table 2) of prostaglandins supports the view that rifamycin SV brought about anti-inflammatory effects. Since prostaglandins are derived exclusively from local synthesis, this implies a reduced synthesis in synovial cells.

In guinea-pig monoarthritis the infiltration of rifamycin SV in affected joints brought about a marked inhibition of RNA synthesis in mixed cell populations which was already detectable 1 h after

infiltration and persisted during the following 24 h (Caruso *et al.*, unpublished). Hence it is reasonable to suppose, on the basis of our clinical and experimental observations, that rifamycin might act simultaneously on the different cellular inflammatory components in man, particularly on RNA-synthesising cells, by directly impeding their growth and reducing the synthesis of mediators responsible for inflammation and its persistence.[27-29] This methotrexate effect was observed in the mononuclear cells of the synovial fluid of rheumatoid and psoriatic arthritis.[10]

The results of our clinical trial indicate that rifamycin SV represents a suitable approach for the local treatment of rheumatoid knee synovitis. Minimal side effects make this treatment particularly acceptable both to the clinician and to the patient.

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