INTRA-ARTICULAR THIOTEPA IN RHEUMATOID ARTHRITIS*

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Scherbel, Schuchter, and Weyman (1957) reported that the local injection of nitrogen mustard directly into rheumatoid joints was safe, and that the injected joints appeared to improve. More recently, Wenley and Glick (1964) at the London Hospital, and Flatt (1962) in the United States have been using triethylene thiophosphamide (Thiotepa, Lederle. Fig. 1), an alkylating agent related to nitrogen mustard, but, not being a vesicant, easier to handle.

after Thiotepa injections into knee joints. However, the dose used in knee joints was limited because of possible systemic toxic effects—the drug is a bone marrow depressant and presumably has mutagenic effects—with the result that a limited dose was acting in a joint with a large synovial surface. It was felt that a more clear-cut result would be obtained using finger joints, allowing a relatively larger dose of the drug to act on the smaller area of synovium. The trial here reported, a continuation of the work begun by Wenley, was designed to assess the effect of single injections of Thiotepa into finger joints and, in a few cases, to follow the changes in synovial histology and fluid following the injections.

ThioTEPA
(Triethylene Thiophosphamide)

Fig. 1.—Thiotepa formula.

Flatt's preliminary report was encouraging, and Wenley and Glick also noted slight improvement

Clinical Trial

Material

Patients were selected with a comparable pair of proximal interphalangeal (PIP) or metacarpo-phalangeal (MCP) joints showing both soft tissue swelling and tenderness. The stage of involvement varied from joints not yet showing erosions to those with extensive changes. Fifty patients completed the trial (with one defaulter). Of these, two were classified as having psoriatic arthropathy, and the remainder "definite rheumatoid arthritis".

Method

One of each pair of joints was selected at random to be injected with a Thiotepa solution prepared by dissolving 15 mg. of the powder in 3 ml. of 1 per cent. procaine solution. Injections were made through the middle of the extensor expansion using a No. 18 needle. The position of the needle within the joint was confirmed by flushing out with procaine, before injecting as much of the Thiotepa solution as the joint would accommodate without undue tension, to a maximum of 1 ml. Doses varied from 0.4 to 1.0 ml. (2 to 5 mg.), average 3.5 mg. The control joint was injected with a similar volume of 1 per cent. procaine solution.

* Based on a paper read to the Heberden Society on December 4, 1964.

The joints were examined before injection and at 2, 6, 10, 16, and 26 weeks. On each occasion the following assessments were made:

- (1) *Pain* on active movement through a full range—graded 0 to 3.
- (2) *Tenderness* on pressure over the joint during passive movement—graded 0 to 3.
- (3) *Range of movement* measured by moulding a strip of soldering alloy to the joint at the extremes of full passive movement, then tracing these angles on to paper.
- (4) *Swelling* assessed visually and, in the case of PIP joints, with jeweller's rings.
- (5) *The patient's assessment* of the relative state of the joints.

Results

MCP joints proved easier to inject than PIP joints, and joints with advanced disease easier than those with earlier involvement. The injection of PIP joints with early involvement was often sufficiently difficult to suggest that this procedure may cause appreciable trauma to the joint cartilage.

There were five occasions at the beginning of the trial when, owing to lack of experience with the technique, doubt was felt about the position of the needle within a PIP joint allocated to Thiotepa, and it was feared that extravasation might jeopardize the digital blood supply. In these cases the allocation was reversed and the opposite joint received Thiotepa. In assessing the results it appears unlikely that this has influenced the final conclusions.

Local reactions following the injections occurred in both groups, with an increase in swelling and discomfort lasting 1 to 10 days. These reactions tended to be more intense and more prolonged in the Thiotepa group, among which 36 (72 per cent.) showed some reaction compared with twenty (40 per cent.) in controls. There were no systemic reactions.

Fig. 2 summarizes the progress of the injected joints during the period of observation. In general it is clear that both those treated with Thiotepa and the control joints have improved by all the criteria used, but that at 26 weeks there is no real difference between the two groups. At 2 weeks the joints given Thiotepa tended to be worse, presumably due to persistence of the initial irritative effect of the drug. The joints receiving procaine, by contrast, were definitely better at this stage. After this the two groups followed the same pattern. There was a steady and statistically significant increase in range

of movement up to 26 weeks, the mean of both groups increasing by about 10°. Ring size, swelling, tenderness, and pain tended to improve up to 16 weeks, then either to remain stationary or to deteriorate slightly.

Fig. 2.—Clinical observations on injected finger joints. Each point is the mean of fifty observations, except for ring size which applies only to the nineteen pairs of PIP joints.

Swelling, tenderness, and pain were graded 0 to 3.

Tables I and II (overleaf) summarize the patient's subjective impressions. On analysis these agree with the clinical findings in showing a definite and identical improvement in both groups. Some bias is apparent in the allocation of joints. Worse joints as judged by the patients, and larger joints as measured by ring size, tending to be allocated to Thiotepa. This is probably due to the changes in random allocation mentioned above. It is thought not to influence the results significantly.

TABLE I
PATIENTS' OBSERVATIONS AT 6 MONTHS

Condition	Thiotepa	Controls
Improved	33	31
Unchanged	16	18
Worse	1	1
Total	50	50

TABLE II
PATIENTS' OBSERVATIONS

Before Treatment	After Treatment		
	T/T Worse	Equal	Control Worse
T/T Worse (24) ..	9	9	6
Equal (14) ..	4	5	5
Control Worse (12) ..	3	5	4

Conclusions

The improvement seen in treated and control joints, by all criteria both subjective and objective, suggests that the joints did in fact improve during the period of observation. The possible causes for this improvement all appear rather unlikely—neither the mechanical trauma of the procedure, nor the chemical effect of the procaine would be expected to have any long-term effect, although it would be of interest to test this. Alternatively, some of these patients did become rather introspective about the injected joints and carried out repeated measurements themselves; it is possible that awareness of the trial and curiosity of this sort may have provoked sufficient exercise of these joints to modify their clinical state. Whatever the explanation it is clear that Thiotepa, as used in this trial, had no advantage over procaine.

Pathological Studies

While the clinical trial was in progress, some pathological studies were undertaken in an attempt to throw light on the mode of action of Thiotepa in rheumatoid joints. These were discontinued when it became apparent that the drug was ineffective clinically, and the study is therefore incomplete. Some of the results are, however, thought to be of sufficient interest to merit reporting briefly.

Histology

Seven rheumatoid patients who were about to undergo surgical synovectomy of finger joints were given a single injection of Thiotepa into one or more of the joints selected for operation. Ten injections were carried out at intervals varying from 3 days to

22 weeks before operation, the non-injected joints acting as controls. At operation two small sections of the synovium were rapidly frozen for immunofluorescent studies, the remainder of the specimen was examined by standard histological methods.

The problem of sampling and the fact that the material was not obtained from the same joints before and after injections make interpretation of the findings difficult, and it is not possible from such a limited number of specimens to form a clear picture of the histological sequence of events following the injections. The one constant change was a reduction in the numbers of inflammatory cells present in the treated synovia. All other features were variable. Some recently injected joints showed considerable surface necrosis, with deposition of extensive sheets of fibrin. About 8 weeks after injection two joints had synovial cell layers reduced to a single thickness of abnormal, vacuolated cells. Below this was rather immature mucoid fibrous tissue. In later sections the synovial cell layer tended to consist of a single layer of almost normal-looking cells with an increase in fibrous tissue more deeply. In general these changes were variable and patchy, with many of the treated joints showing areas which appeared to have been unaffected by the drug. An overall reduction in inflammatory cells was, however, seen in all treated joints. Some of these changes are illustrated in Figs 3, 4, and 5 (opposite).

Immunofluorescent studies were carried out on most of these specimens in an attempt to find out whether Thiotepa modified the numbers of rheumatoid factor containing cells present—for it has been suggested by Flatt (1962) that cytotoxic drugs may act by suppressing local immune processes. The technique described by McCormick (1963) was followed, the sections being exposed to fluorescein-labelled heat aggregated human gamma globulin. In only two sero-positive cases were fluorescent cells demonstrated in any numbers and in neither of these was there any appreciable difference in the number of fluorescent cells in the treated as compared to the control joint—despite a striking difference in the cell numbers judged by standard histology on the same specimens. This scanty information would suggest that populations of rheumatoid factor containing plasma cells are, if anything, more resistant to the effect of Thiotepa than are lymphocytes.

Synovial Fluid Studies

A limitation imposed by studying finger joints is that not enough synovial fluid can be obtained for examination. So six rheumatoid patients, each with

Figs 3, 4, 5.—Three pairs of synovial sections ($\times 100$) from (a) non-injected controls, and (b) joints injected with Thiotepa. (Each pair from one patient.) These fields were selected to emphasize the contrast between treated and untreated synovia. Changes in treated joints were variable and patchy.

(3b). 4 weeks after injection; area showing reduction in cellularity and replacement with mucoid-oedematous fibrous tissue.

(4b). 8 weeks after injection; fibrin sheet overlying mucoid regenerating fibrous tissue.

(5b). 10 weeks after injection; surface fibrin and deeper fibrous tissue.

positive rheumatoid serology and a chronic knee effusion, were selected for this part of the investigation. Each knee was aspirated, then injected with a single relatively large (30-mg.) dose of Thiotepa; 5 to 8 weeks later these joints were re-aspirated. Blood specimens were taken on each occasion.

Fig. 6 shows the titre of rheumatoid factor (latex-fixation test) in these specimens. An interesting result to emerge was a consistent fall in the titre of rheumatoid factor not only in the synovial fluid but in the serum. A similar result has been reported by Gross (1963), although Zuckner, Uddin, Ramsey, Gantner, Ahern, and Dorner (1964) found no such changes. The limited number of tubes used in these tests was insufficient to record some of the higher titres—nevertheless the trend seems definite, with a fall in titre of approximately three tubes in both fluids. Protein determinations carried out on the same specimens suggested that overall protein changes were not responsible for this fall in titre (Fig. 6).

In an effort to document these changes more precisely immuno-electrophoresis was carried out on all specimens. The findings in all the fluids were very similar and two examples are shown in Fig. 7 (opposite); in contrast with normal synovial fluids these specimens showed a marked increase in all three immunoglobulins, which is essentially unchanged by Thiotepa treatment.

Summary and Conclusions

Intra-articular injections of Thiotepa into rheumatoid finger-joints produced some clinical improvement which was still apparent after 6 months. Control joints injected with procaine showed a similar improvement.

Histologically, Thiotepa reduced the numbers of inflammatory cells in the treated synovia, while intra-articular injections of larger doses into knee joints produced a fall in rheumatoid factor titre both in the synovial fluid and in the blood.

Fig. 6.—Changes in latex-fixation titre in serum and synovial fluid of knee joints after intra-articular injection of 30 mg. Thiotepa. Below: Total protein and globulin estimations in the synovial fluid specimens.

Fig. 7.—Immunoelectrophoretic patterns of synovial fluids before (A and X) and 6 weeks after (B and Y) intra-articular injection of 30 mg Thiotepa. Rabbit anti-human serum used. N = normal joint fluids.

Despite the theoretical attractions of administering cytotoxic drugs directly into rheumatoid joints, and the marked histological changes which this has been shown to produce, it is concluded that injecting Thiotepa into rheumatoid finger joints, as carried out in this trial, is not a worth-while procedure.

I am indebted to a number of people for assistance. Professor R. G. White supervised the immunofluorescent studies and Dr. P. Wilkinson carried out the immunoelectrophoresis. Dr. J. Landells reported on all the histological sections, which were obtained at operations carried out by Mr. O. Vaughan-Jackson. Dr. J. M. McCormick and Dr. W. G. Wenley gave helpful advice. Miss Wendy Grant kindly undertook the statistical analysis. The patients were under the care of either

Dr. W. S. Tegner or Dr. R. M. Mason, to both of whom I owe thanks. Messrs Lederle kindly provided the Thiotepa.

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Discussion

DR. B. M. ANSELL (*Taplow*): Have you made any histological studies of metacarpophalangeal joints after steroid injections? In the knee there is a decrease in inflammatory cells.

DR. CURREY: Only one, in which we used steroid injection as a control. This was one of those showing no dramatic change.

DR. W. G. WENLEY (*Norwich*): These results are disappointing but I should not write off Thiotepa yet. It might be worth repeating the trial using saline as a control instead of procaine. I am still using Thiotepa—it is useful in knees, where it can dry up effusions which have failed to respond to aspiration and cortisone.

DR. V. WRIGHT (*Leeds*): You have shown convincingly that both Thiotepa and procaine produce improvement in some patients. Did the absolute numbers of these exceed the one-third of placebo reactors who might be anticipated to improve?

DR. CURREY: Of the fifty patients in each group, 33 and thirty respectively claimed subjective improvement.

PROF. J. H. KELLGREN (*Manchester*): The most interesting feature here is the dissociation between striking histological improvement and absence of clinical improvement. Have you any comments?

DR. CURREY: No. Perhaps procaine does relieve pain and allows better joint movement. This would as it were dilute the observed improvement seen with Thiotepa.

DR. K. W. W. H. WALTON (*Birmingham*): Dr. David Palmer recently studied the effect of Thiotepa on the granulation tissue of experimental arthritis in rabbits and guinea-pigs. He found that injections of Thiotepa produced acute necrosis of guinea-pig tissue, but that the overall effect on carrageenan arthritis was very little different in animals having injections and those without.

DR. J. H. GLYN (*London*): Dr. Flatt is now using osmic acid. Why did he give up Thiotepa?

DR. CURREY: I have not used osmic acid. Early favourable reports of osmic acid have not been followed by any controlled trial, which suggests that later results may have been disappointing.

PROF. J. H. KELLGREN (*Manchester*): Osmic acid was extensively used 15 years ago!

DR. E. N. GLICK (*London*): A single dose of 15 mg. Thiotepa into the knee joint is not very effective and as about 25 per cent. of patients have a transient leucopenia one cannot really increase the dose. Repeated injections seem to produce some benefit and no more leucopenia. In animals 0.1 mg. per kg. given systemically increases

the cellular mutation rate. Leucopenia is evidence of systemic absorption from the knee joint—if half of a 15 mg. dose reaches the blood-stream the gonads or marrow may be affected, so it should not be given to patients in the reproductive period.

A SPEAKER: What was the reason for selecting Thiotepa?

DR. CURREY: It is safe to handle—injections into joints are always put in under pressure with the risk of getting some on the skin or into one's eye. It is not a vesicant like nitrogen mustard.

Thiotepa intra-articulaire dans l'arthrite rhumatismale

RÉSUMÉ

Des injections intra-articulaires de Thiotepa dans les articulations rhumatismales des doigts ont produit une amélioration clinique qui était encore apparente six mois plus tard. Des articulations dans lesquelles on avait injecté de la procaine à titre de comparaison ont accusé une amélioration similaire.

Du point de vue histologique, Thiotepa réduisit le nombre des cellules inflammatoires dans la synoviale traitée; des injections intra-articulaires de doses plus fortes dans les articulations des genoux amenèrent une chute du taux du facteur rhumatisal aussi bien dans le liquide synovial que dans le sang.

Malgré l'attrait théorique des produits cytotoxiques injectés directement dans les articulations rhumatismales, et des altérations histologiques prononcées démontrées ici, l'injection de Thiotepa dans les articulations rhumatoïdes des doigts, de la manière effectuée dans cet essai, n'est pas à recommander.

Thiotepa intra-articular en la artritis reumatoide

SUMARIO

Inyecciones intra-articulares de Thiotepa en articulaciones reumatoides de los dedos produjeron una mejoría clínica que aun fué aparente seis meses después. Articulaciones de control inyectadas con procaina acusaron una mejoría semejante.

Desde el punto de vista histológico, Thiotepa redujo el número de células inflamatorias en la sinovia tratada; además, inyecciones intra-articulares de más fuertes dosis en la articulación de la rodilla ocasionaron una caída de las cifras del factor reumatoide tanto en el líquido sinovial como en la sangre.

A pesar de la atracción teórica de los productos citotóxicos administrados directamente en las articulaciones reumatoides, y de las alteraciones histológicas pronunciadas, demostradas aquí, inyecciones de Thiotepa en las articulaciones reumatoides de los dedos, del modo efectuado en esta investigación, no se recomiendan.