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Treatment of Hemophilic Arthritis With D-Penicillamine: A Preliminary Report

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Current medical management programs for established joint diseases in hemophiliacs are unsatisfactory and do not modify the eventual outcome. D-penicillamine, a drug effective in the proliferative synovitis of rheumatoid arthritis, was evaluated in a rabbit model of hemarthroses-induced arthritis and in four hemophiliacs with chronic synovitis. The animals had intra-articular injections of citrate (left knees) and autologous citrated whole blood (right knees). Eight weeks later, the rabbits were divided into two groups: no treatment and D-penicillamine (50 mg/kg/day, IM) until sacrificed at 6 months. The saline-injected joints showed no inflammation and no iron deposition. The blood-injected knees showed iron deposition in both groups, the D-penicillamine animals had marked suppression of chronic inflammation. Of the four patients treated, three had clinical responses (reduction in synovial thickness, reduction in number of bleeds in the affected joint). One patient, who did not respond, developed mild-moderate proteinuria. Those patients who responded received between 5.3 and 7.1 mg/kg/day of the drug. Mild abnormalities in platelet aggregation were seen in the responders. This preliminary study suggests that D-penicillamine is beneficial in the chronic synovitis/arthritis induced by hemarthroses. Further trials are recommended.

Key words: hemophilia, synovitis, rabbits, D-penicillamine

INTRODUCTION

Current management programs for hemophilia have greatly reduced the frequency of acute and chronic joint disease in these patients. However, a significant number eventually develop at least one joint with chronic synovitis or arthritis during childhood [1,2]. Inability to medically control the joint disease at this stage allows for repeated hemarthroses and subsequent joint destruction, contractures, and ankylosis. Medical management of established arthritis/synovitis is unsatisfactory and includes oral or intra-articular corticosteroids, nonsteroidal anti-inflammatory drugs (indomethacin, ibuprofen, choline magnesium trisalicylate), chemical synovectomy with osmic acid, and prophylactic administration of the missing procoagulant [3–8].

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Although drug therapy has been reported to be effective in temporarily controlling the symptoms and signs of hemophilic arthritis, it does not appear to modify the eventual outcome.

The gross and microscopic similarities of early proliferative synovitis of hemophilic arthritis and rheumatoid arthritis are striking [9–14]. D-penicillamine is a drug with anti-inflammatory activity that is effective in children with rheumatoid arthritis [15–16]. Penicillamine is classified as a disease-modifying antirheumatic drug, as are gold-containing compounds, cytotoxic drugs, and the antimalarials, because it is believed that it may influence the underlying course of rheumatoid arthritis [17]. In view of its effectiveness in the synovitis of rheumatoid arthritis, we studied the effect of D-penicillamine in a rabbit model of hemarthrosis-induced arthritis and subsequently in hemophilic patients with synovitis. In this preliminary study we found it to be effective and without significant hematologic side effects.

MATERIALS AND METHODS

Rabbit Experiments

The procedure to produce hemarthrosis-induced arthritis was a modification of the method of Wolf and Mankin [18]. New Zealand white rabbits (1.0 to 1.5 kg body weight) had intra-articular injections of citrated autologous blood into the right knee and citrated saline into the left knee; 2.5 ml 3 days a week for 2 weeks and then 2.5 ml 2 days a week every other week for 6 months. A total of 27 animals were used. The time of appearance of synovitis/arthritis was established as 8 weeks using six rabbits that were killed and examined, grossly and histologically, at monthly intervals. The remaining rabbits were divided into no-treatment (11 animals) and penicillamine (10 animals) treatment groups. D-penicillamine (a gift from Clement Stone, Ph.D., Research Laboratory, Merk Sharp and Dohme, West Point, PA) was given as 50 mg/kg/day intramuscularly. (Sterile D-penicillamine solution contained 50 mg of the drug and 0.05 mg edetate disodium per ml, pH 6.5.) The animals were killed at 6 months. Both knees of all animals were opened immediately postmortem and tissues were photographed. Suprapatellar synovium was removed and fixed with 10% neutral formalin, embedded in paraffin, and sectioned. Tissue sections were stained with H & E and examined for synovial lining cell hyperplasia, amount and location (diffuse, focal, or perivascular) of polymorphonuclear and lymphocytic infiltration, and Gomori stain for the amount and location of iron deposition. Each slide was graded, 0 to 4+, except for synovial lining cells. Synovial lining cells represent the number of cells lining the synovium. The Chronic Inflammatory Index was calculated by multiplying the graded chronic (mononuclear cells) inflammatory response (0 to 4+) by the percent of synovium involved. The histologic specimens were coded and read in a blind fashion by two of us (K.K., E.P.G.) and an independent veterinary pathologist. An objective and reproducible scoring system for grading synovitis that has been described was employed [19].

Human Studies

Hemophiliacs, enrolled in the Mountain States Regional Hemophilia Center in Tucson, with physical signs of synovitis (palpable synovium, with or without effusion) unresponsive or poorly responsive to steroids were studied. Initial and follow-up joint examinations were performed by the rheumatologist (K.K.), physical therapist, hem-

ophilia center nurse (M.L.D.), and center director (J.J.C.) on all cases. X-rays of affected joints were obtained at the beginning of the study in order to radiologically stage the joint disease [20]. No patient was receiving concomitant corticosteroids or nonsteroidal anti-inflammatory drugs, or prophylactic factor infusions. All patients had complete blood counts and urinalysis performed monthly while on penicillamine therapy. Also, platelet aggregation tests (using ADP, collagen, and epinephrine) were done initially, first at 1 month and then every other month. Bleeding times were not done. D-penicillamine was prescribed in the dose of 2–7 mg/kg/day in one oral dose in the morning before breakfast. No patient received more than 10 mg/kg/day. Consent was obtained from each patient and his parents. The study was approved by the Human Subjects Committee, University of Arizona.

RESULTS

Rabbit Study

The number of synovial lining cells (SLC), amount of iron deposition, and the magnitude of chronic inflammation (mononuclear cell infiltrate) in animals treated and not treated with penicillamine are shown in Table I. Penicillamine therapy was not associated with a change in SLC or iron deposition. There was a 44% reduction in chronic inflammation compared to the hermarthrosis nonpenicillamine-treated animals (called "blood control" in Table I). Figure 1 shows the inflammatory index for these rabbits. This index is calculated by multiplying the chronic inflammation value by the percent of synovium involved with inflammation. The penicillamine-treated animals had a significant reduction in this index when compared to untreated controls ($p < 0.05$). Representative histologic findings, using H & E stain, are shown on Figures 2–4. Figure 2 shows the appearance of the synovium in the knee injected with citrate only. Figure 3 shows injection of autologous whole blood without treatment and Figure 4 shows injection of autologous whole blood plus IM penicillamine therapy.

Human Studies

Four patients with severe hemophilia A (< 1 unit/dl of factor VIII), (one with a factor VIII inhibitor [Patient 3]), with chronic synovitis were studied. Penicillamine was given in a dose range of 2.4 to 7.1 mg/kg/day for 1 to greater than 12 months (Table II). Three patients had a definite unequivocal response as judged independently

TABLE I. Rabbit Data*

	N	SLC (Nos.)	Chronic inflammation (0–4+)	Iron (0–4+)
Blood control	11	3.41 ± 0.3	1.41 ± 0.23	2.36 ± 0.24
Saline control	11	1.90 ± 0.77	0.50 ± 0.15**	0.22 ± 0.08**
Blood penicillamine	10	3.04 ± 0.27	0.79 ± 0.19**	1.83 ± 0.11

*N, number; SLC, synovial lining cell.
**$p < 0.05$.

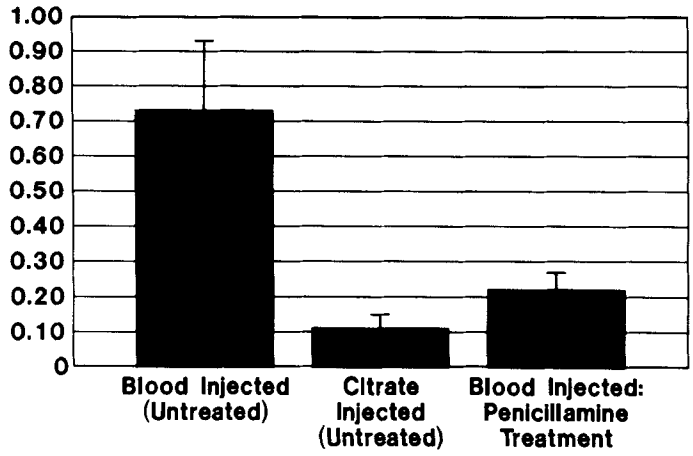


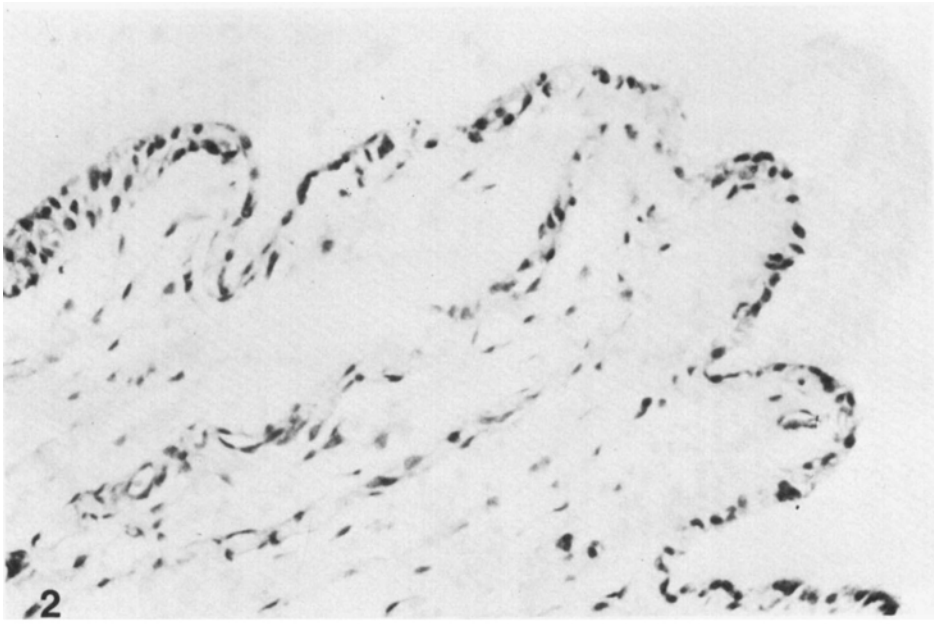
Fig. 1. Synovial chronic inflammation index (graded chronic inflammatory response multiplied by the percent of involved synovium). Significant reduction in the index in animals with hemarthrosis treated with D-penicillamine compared to no treatment (blood control).

by four observers. There was reduction in synovial thickening in Patient 3 and disappearance of the palpable synovium in Patients 1 and 2. Patient 1 was experiencing recurrent hemarthroses in the left knee. Between bleeding episodes the joint was swollen, boggy, and warm. He had poor response to courses of prednisone and with casting. Within 6 weeks of penicillamine therapy (125 mg/day) the palpable synovium was less obvious but he had had five bleeding episodes in the joint. Between 6 and 10 weeks of therapy the knee was without swelling, there was no palpable synovium or fluid, and he had had no bleeding in that joint. The penicillamine was discontinued after 10 weeks. He continues to do well 1 year later.

Patient 2 had chronic synovitis and recurrent hemarthroses of both ankles (R > L); six bleeds into the left and eight bleeds into the right per year. One month after the initiation of penicillamine (250 mg/day) he had had two right-ankle hemarthroses and one left-ankle bleed with definite clinical evidence of a reduction in the synovial thickening on the right. After 6 months, he has had no bleeding into the left ankle and four into the right. Both ankles have reduction in palpable synovium (left is absent; right is minimally felt). He continues on the drug.

Patient 3 has chronic synovitis plus the findings of stage IV disease in both knees. He also has a factor VIII inhibitor and is usually given prothrombin complex concentrates for bleeding episodes. While receiving 125 and 250 mg/day of penicillamine there was no reduction in bleeding episodes or clear evidence of reduction in the thickened synovium. (He had six bleeds into the right knee and four into the left over a 9-month period.) During the 4-month period of receiving 375 mg/day of the drug he has had no bleeds into the right knee and two into the left. The synovial thickening has lessened but not disappeared. He continues on the drug.

Patient 4 has recurrent hemarthroses and chronic synovitis of both ankles. No change in his bleeding episodes or joint exam occurred during the 8 months of penicillamine therapy. His course was complicated by the appearance of moderate proteinuria (1.1 gm/24 hr) at 6 months while receiving 250 mg/day of penicillamine. The dose was reduced to 125 mg/day but he continued to have 2+ proteinuria. After a total of 8 months the drug was discontinued. Although the proteinuria persists, it is



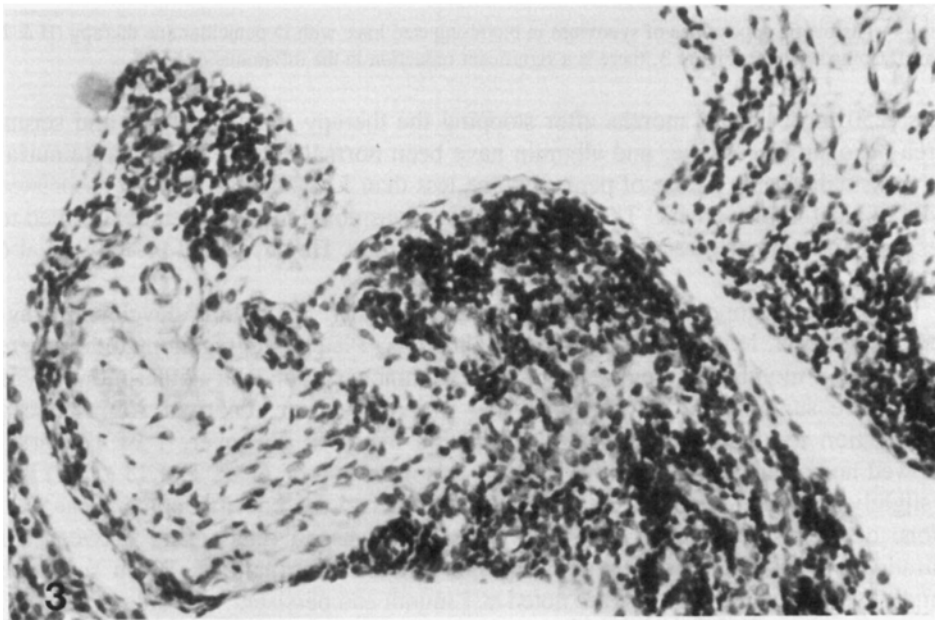


Fig. 2. Histologic appearance of synovium in citrate injected knee (H & E stain). Note lack of inflammatory response.

Fig. 3. Histologic appearance of synovium in blood-injected knee and no D-penicillamine (H & E stain). Note marked inflammation.

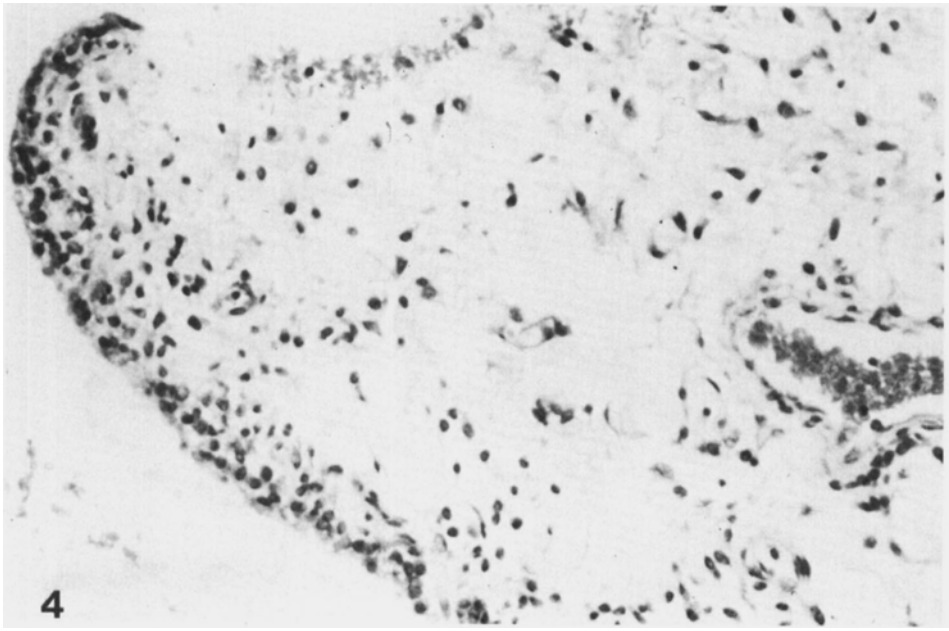


Fig. 4 Histologic appearance of synovium in blood-injected knee with D-penicillamine therapy (H & E stain). Compared with Figure 3, there is a significant reduction in the inflammation.

less (250 mg/24 hr) 4 months after stopping the therapy. His urinalyses and serum urea nitrogen, creatinine, and albumin have been normal except for the proteinuria. In this small series, a dose of penicillamine less than 5 mg/kg/day was not associated with clinical improvement. The only side effect was noted in the patient who failed to respond and who also received less than 5 mg/kg/day. He developed proteinuria at 6 months.

Complete blood counts were performed monthly. No patient developed drug-induced anemia, leukopenia, or thrombocytopenia. Platelet aggregation studies were performed 1 month after beginning D-penicillamine and then every other month. The results are summarized on Table III. No patients had an abnormal ADP-induced aggregation test with the higher concentration of ADP. However, 7 of 13 (54%) showed no secondary wave with the lower concentration of ADP, 3 of 13 (23%) had a slightly impaired collagen release, and one (8%) had an abnormal epinephrine test. None of these abnormalities were severe. There was no relationship between the duration of penicillamine treatment and the platelet abnormalities. When seen, the mildly reduced aggregations were noted at 1 month and persisted, and did not worsen, during the course of treatment. There was no difference between 5 or 7 mg/kg/day drug dosage. However, less than 5 mg/kg/day was associated with normal platelet function. There was not an increased frequency of bleeding in other sites while the patients were receiving the drug.

DISCUSSION

The proliferative synovitis in hemophilia is characterized by synovial hyperplasia, acute and chronic inflammatory cells, and hemosiderin deposits [9,10,12–14].

TABLE II. Severe Hemophilia Patients With Synovitis

Patient	Factor deficiency	Age (years)	Weight (kg)	Penicillamine Dose mg/day	mg/kg	Duration	Joint and stage[a]	Response	Side effects
1	VIII	7	18.6	125	6.7	10 Weeks	Left knee (II)	Yes	None
2	VIII	13	47	250	5.3	> 6 Months	Left ankle (II) and Right ankle (III)	Yes	None
3	VIII	17	53	125	2.4	1 Month	Right knee (IV)	No	None
				250	4.7	8 Months	and	Undecided	None
				375	7.1	> 4 Months	Left knee (IV)	Yes	None
4	VIII	10	52	250	4.8	7 Months	Right ankle (II)	No	Proteinuria
				125	2.4	1 Month		No	

[a]Radiological stage.

TABLE III. Platelet Aggregation (Mean and Range)*

	ADP 23 mM	ADP 11.5 mM	Collagen	Epinephrine
Patients before drug (4)	> 70%	> 60%	> 70%	> 70%
With drug (15)	75%, 61-94	53%, 25-84	71%, 61-87	75%, 51-94
Normal controls	> 60%	> 50	> 70	> 60

*Number of determinations are in parentheses.

The cause appears to be due to joint irritation caused by repeated, chronic hemarthrosis. It is not known if hemophilic arthritis may also be mediated by immune mechanisms. Penicillamine has many affects and under certain circumstances has been found to have an immune suppression action, an anti-inflammatory effect, and to be a strong chelator [17]. In our rabbit model the drug's chelating ability for iron was found not to be a major mechanism of action. There was no change in the histologic grading of iron deposition in penicillamine-treated rabbits compared to the nonpenicillamine group. Also, there was no effect on the synovial cell layer. However, there was a marked reduction of the chronic inflammatory index in the drug-treated group. Although no immunologic studies were performed in the rabbits or the hemophilic cases the anti-inflammatory action of the penicillamine seems to explain best the resolution of the arthritis.

The frequency of intra-articular bleeding in the affected joint decreased during the time the patients were receiving the drug and when the joint swelling was resolving. This is consistent with the pathogenesis of the process as described by Sokoloff and colleagues, in that patients with hemophilic synovitis tend to have repeated episodes of clinical and subclinical hemarthrosis in these affected joints [11]. This study used physical examination to detect synovial thickening and changes in synovial mass. This is, admittedly, a crude estimate.Using the joint circumference was not found to be useful because of the bony overgrowth in those patients with Stage III and IV disease and the presence of intra-articular fluid. We did not feel that repeated invasive procedures, such as arthroscopy, were justified in this preliminary study. At the present time there are no published reports on how accurately to assess synovial size or change by noninvasive techniques. In our study, we used four independent observers, skilled in assessing joint disease, to minimize bias.

D-penicillamine is chemically a structural analogue of cysteine. It is a strong chelator, especially for copper (Cu^{++}), and is used for this purpose in Wilson's disease. Although it is not known by what mechanism penicillamine acts in certain rheumatic diseases, recent studies suggest that the penicillamine-copper complex has potent anti-inflammatory activity [21,22]. This complex appears to have superoxide-dismutase activity [23]. Perhaps its anti-inflammatory activity is related to it being a free radical scavenger.

There are numerous side effects (20–30% of cases) that can occur with D-penicillamine [17,24]. Most are dose related. We have found that in our nonhemophilic patients a dose that does not exceed 10 mg/kg/day is safe and rarely associated with significant toxicity. Penicillamine can and has been used safely in patients with

penicillin allergy [25]. One of our patients experienced a penicillamine-induced side effect, ie, proteinuria. Drug-induced proteinuria occurs in up to 20% of patients receiving long-term D-penicillamine therapy. Its frequency appears to be dose and duration related. However, the published toxicity data reveal extreme variability among patients. The proteinuria, usually unassociated with other urinary abnormalities, is thought to be due to immune complex disease. It disappears with discontinuation of the drug but can persist for over a year. Although renal abnormalities have been described in multiply infused hemophiliacs, the occurrence of isolated proteinuria is unusual [26]. Thus, in our one case it would appear that the mild-moderate proteinuria was probably related to the drug. Penicillamine has not been reported to alter platelet functions but we noted mild abnormalities in platelet aggregation when the dose of the drug was $\geqslant 5$ mg/kg/day. There was not an increased frequency of bleeding in other sites while the patients were receiving the drug. Nonetheless, penicillamine appears to be an effective drug in hemophilic synovitis. These preliminary studies suggest that controlled studies are needed to evaluate frequency of effectiveness, dose response relationship, frequency of drug-induced toxicity, and long-term effectiveness in possibly reducing or eliminating progressive joint disease in hemophilic arthropathy.

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