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The Evaluation of Natural Substances in the Treatment of Adjuvant Arthritis*

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The podiatrist is often called upon to treat rheumatoid arthritis, a potentially disabling disease. Many current treatments have dangerous side effects, and safer alternatives must be found. Adjuvant-induced arthritis in rats which closely resembles rheumatoid arthritis was used as the experimental model. The naturally occurring nonsteroidal substances – ascorbic acid, thymus extract, aloe extract, and deoxyribonucleic acid – showed varying degrees of effectiveness in reducing the disease. Aloe extract was the most potent anti-inflammatory agent, while ascorbic acid proved to be the most effective immunosuppressive agent. This work may provide effective treatment modalities for preventing and treating rheumatoid arthritis.

Rheumatoid arthritis, a chronic systemic disease, is characterized by inflammation of peripheral joints.[1] Adjuvant arthritis in rats closely resembles rheumatoid arthritis in its pathologic and clinical manifestations, and provides an excellent experimental model.[2–4] The induction of adjuvant arthritis by injection of *Mycobacterium butyricum* in mineral oil is well known.[5,6]

Adjuvant arthritis results from an immunological response to antigen present in the *M. butyricum* capsule amplified by the oil adjuvant. The mycobacterial antigen, recognized as foreign by the host immunological system, starts a stereotyped series of responses.[5,6]

At the site of injection, denaturation of collagen occurs, which acts as an antigen and results in the stimulation of autoantibodies. These autoantibodies then circulate throughout the body, attacking normal collagen.[7,8]

The induced arthritis which develops in the injected paw represents a primary inflammatory reaction, whereas the swelling in the noninjected contralateral paw represents a systemic immunological response.[4,9]

Therapeutic agents against adjuvant arthritis can be divided into preventive agents that reduce the primary inflammation at the injection site, and immunosuppressive agents that prevent the systemic immunological disease. Immunosuppressive agents must reduce the disease in the noninjected paws.[9–11] We feel several substances must be considered for testing because of their possible anti-arthritic properties.

Thymus dependent T cells function in cell-mediated immunity allowing for delayed hypersensitivity reactions in the body, and the development of secondary lesions in polyarthritis. Ziff[12] mentioned that the cellular and humoral limb of the immune response were activated in the rheumatoid synovium. His research suggests that interaction of T and B lymphocytes occurs with liberation of

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lymphokines that stimulate B lymphocytes to antibody synthesis. We feel, therefore, that thymus extract inoculations could have an effect on the development of rheumatoid arthritis, especially on the secondary lesion.

Ascorbic acid intrigues us as a possible anti-arthritic agent. Abnormal collagen forms in the rheumatoid arthritic synovial tissue.[13] Ascorbic acid plays an essential role in the formation of collagen by hydroxylation of proline to hydroxyproline.[14, 15] We believe, therefore, that high levels of ascorbic acid in adjuvant-diseased rats might prevent excessive collagen breakdown. High levels of ascorbic acid naturally found in the adrenal cortex could serve to hydroxylate the corticosteroid hormone.[14] Corticosteroid stimulation by ascorbic acid could have a delayed suppressing effect on secondary inflammation. DNA (deoxyribonucleic acid) may serve a possible anti-inflammatory duty by alleviating the development of the primary inflammatory lesion in polyarthritis. RNA (ribonucleic acid) has an anti-inflammatory effect, both on edema associated with adjuvant arthritis and gelatin-induced inflammation in mice.[16, 17] Since DNA is the template upon which RNA is transcribed, DNA could display anti-inflammatory activity.

Immunosuppressive steroids can inhibit DNA synthesis and mitosis and suppress inflammation.[18–20] We feel that a DNA produced exacerbation of the immune response might indicate the area of the control mechanism by which the anti-inflammatory glucocorticoids act.

The cosmetic industry has demonstrated over the years the possible therapeutic activity of aloe (*Aloe africana*); therefore, we wanted to test this substance in our experimental model. For centuries, people of the Caribbean have treated the inflammation of eczema, burns, and insect bites with aloe. Aloe could act as a nonsteroidal anti-inflammatory agent.

By measuring the edemic paw volume of adjuvant arthritic rats, we will determine disease severity and evaluate the anti-inflammatory and immunosuppressive activity of these substances.

The objective of this study is to compare ascorbic acid, thymus extract, aloe extract, and DNA in adjuvant arthritic rats as potential therapeutic agents for podiatry in treatment of rheumatoid arthritis.

Materials and Methods

Adult male Sprague-Dawley rats (175–225 g; 10–12/group) were housed with 12-hr light cycles and fed commercial chow. Heat-killed *M. butyricum* was suspended in light mineral oil, making a final concentration of 5 mg/ml.

Under ether anesthetic, each rat was injected on the plantar aspect of the hind paw with either 0.1 ml of oil or 0.1 ml of *M. butyricum* in oil into the right paw. The initial time of injection was designated day 0. Our study consisted of two separate experiments; one studying prevention of the disease state, while the other will follow the regression of the already established arthritis. Hind paw volumes were used to monitor the disease state. Two sets of controls were used for each study. One served as an oil control while the other served as an adjuvant control. Neither the oil or adjuvant controls received any treatment and served as references for determining the therapeutic effects of ascorbic acid (sodium salt dissolved in water), thymus extract (homogenized bovine tissue dissolved in water), aloe plant extract (leaves homogenized and suspended in water (*A. africana*)), and DNA (sodium salt from herring sperm (type VII) dissolved in water). Differences in paw volume between the adjuvant control rats and the experimentally treated rats were attributed to the effects of the injected agents.

In the prevention study, rats were injected with *M. butyricum* in oil as described, and then separated into five groups. One group served as a control. The other groups received 150 mg/kg daily subcutaneous injections of ascorbic acid, thymus extract, aloe plant extract, or DNA, beginning on day 0 and ending on day 12 (13 days of treatment).

In the regression study, the rats were injected with *M. butyricum* in oil and set aside until the classic symptoms of adjuvant arthritis developed. These events usually occurred from 14 to 21 days after administration of adjuvant.[4] Treatment began on day 21 and continued through day 33 (13 days of treatment). The treatment plan for the regression study was identical with the prevention study, but there was an initial delay of 21 days.

A mercury plethysmograph was used to monitor the hind paw volume. The plethysmograph was zero prior to each reading.

Mycobacterial injections were made into the right hind paw and edema in this paw was viewed as an inflammatory response. We assumed that inflammation would be prolonged because of the long lasting antigen levels maintained by the slow bacterial release from the mineral oil into the tissue. Any edema in the left paw was thought to be the result of an immunologic phenomenon, as this paw was not subjected to trauma.[10, 11] We expected increased edema in the injected paw as a result of progression of systemic disease.

The rats were anesthetized with ether on mea-

suring days, and hind paw volume was determined by dipping the hind paw in a mercury-filled cell up to its anatomic hair line. The initial measurements were taken 6 hr after injection of *M. butyricum* in mineral oil or oil alone. Day 0 served as a reference from which units of edema were measured in the prevention study. Units of edema were calculated by subtracting the day 0 volumes from those measured on days 7, 14, and 21. The baseline values for calculating daily changes in edema utilized day 21 measurements. All day 21 volumes were viewed as zero units of edema. The hind paw volumes for this aspect of the study were determined on days 0, 21, 28, 35, and 38.

Hind paws were severed at the anatomic hairlines and weighed at the end of each study. In order to rule out any variation in paw volume caused by body weight, the relative paw volumes were calculated. This was performed by expressing the hind paw weight/total body weight × 100. Using a CU-5 Medical Land Camera,[®1] representative rats were photographed from both groups on days 0, 21, and 38 to demonstrate the difference in paw edema between injected and noninjected paws. In addition, xerographs were taken to demonstrate marked soft tissue and osseous changes. Standard errors were determined using the formula:[21] $SE = -\sqrt{d^2/n(N-1)}$.

Results and Discussion

Prevention Study

Adult male Sprague-Dawley rats were inoculated with *M. butyricum* in mineral oil (0.5 mg/0.1 ml) on day 0 to monitor preventive effects. *M. Butyricum* produced increased swelling over 21 days in the left and right paw. The left paw increased from 0.9 ± 0.42 to 14.69 ± 3.98 whereas the right paw volume increased from 1.14 ± 0.7 to 23.22 ± 4.9. Treatment with preventive agents began immediately after injection and terminated 12 days later. Doses were 150 mg/kg of body weight.

The data concerning ascorbic acid, thymus extract, aloe extract, and DNA are represented graphically for the reader's comparison. These figures and tables compare effects between injected and noninjected paws.

On day 7, with DNA, aloe, and ascorbic acid, an early trend was noted toward inhibition of edema in the noninjected paw. Only aloe showed edema inhibition in the injected paw. However, by day 14, aloe produced a pronounced inhibition in both paws. On day 21, aloe produced the most radical edema reduction in both paws. Only aloe showed a continued trend in edema reduction in both paws. Inhibition was 72 and 48% within the noninjected and injected paws, respectively. This was the most significant reduction in edema observed within the prevention study. This result suggests that aloe could be used by the podiatrist for the alleviation of edema within the acute inflammatory phase of rheumatoid arthritis (Table I, Figs. 1 and 2).

However, all the agents showed a significant decrease in edema in both the injected and noninjected paw by day 21. Thymus produced a 67% inhibition and DNA a 62% inhibition, both in the noninjected paw. The injected paw inhibitions were 16 and 32%, respectively. Aloe, DNA, and thymus exhibited a threefold, twofold, and onefold inhibition of edema (48, 32, and 16%, respectively) (Table I.) Ascorbic acid showed the least effect on inhibition of edema in the noninjected and injected paws, respectively, 29 and 0.86%.

Ascorbic Acid Prevention

Administration of 150 mg/kg of ascorbic acid over 13 days inhibited paw volume by 29% over 21 days compared to controls (Table I, Figs. 1 and 2.) Abnormal collagen is present in the rheumatoid arthritic's synovial tissue.[13] Mycobacterial antigen stereotypically induces the rat's immunological system to break down connective tissue in the area of injection. This denatured collagen then acts as "self" antigen stimulating the production of autoantibodies. These immunoglobulins circulate throughout the body attacking normal collagen and play a role in the inflammatory process. Subsequent formation of antigen-antibody complexes then occurs with release of histamine and other inflammatory mediators such as kinins, seratonins, and slow-reacting substance of anaphylaxis.[5, 6]

Any factor that helps in the synthesis of collagen could possibly counterbalance collagen breakdown and, subsequently, the development of autoantibodies. Many studies have shown that ascorbic acid enhances the development of collagen. Ascorbic acid promotes aggregates of ribosomes in the endoplasmic reticulum to facilitate collagen synthesis.[5, 22] Incubation of human synovial cells with pharmacologic concentrations of ascorbic acid stimulate accumulation of fibrillar collagen.[23] Embryonic human lung fibroblasts depend on ascorbic acid for full hydroxylation of collagen.[24] Ascorbic acid also plays an essential role in the formation of collagen by hydroxylation of proline to hydroxyproline.[14, 15] High levels of ascorbic acid are found in

[®1] Polaroid Corporation, Cambridge, MA.

Table 1. Prevention of Adjuvant Arthritis with Ascorbic Acid, Thymus Extract, Aloe Extract, and DNA

	Adjuvant	Ascorbic Acid	Thymus Extract	Aloe Extract	DNA
Treatment (mg/kg × 13) day 21–33		150	150	150	150
Number of rats	11	12	12	12	12
Final body weight (grams)	376 ± 20	291 ± 11	307 ± 9	317 ± 7	294 ± 11
		Edema of hind paws (volume units ± SE)			
Day 7					
Left	0.90 ± 0.42	0.49 ± 0.3	1.68 ± 0.3	0.43 ± 0.6	0.00 ± 0.5
Right	1.14 ± 0.70	2.50 ± 0.8	2.37 ± 0.6	0.50 ± 0.7	1.83 ± 1.0
Day 14					
Left	3.45 ± 1.40	3.93 ± 1.0	7.08 ± 0.6	1.34 ± 0.6	4.10 ± 1.0
Right	9.95 ± 1.50	9.87 ± 6.0	12.09 ± 2.0	4.57 ± 0.9	8.61 ± 2.0
Day 21					
Left	14.69 ± 3.98	10.43 ± 3.0	4.79 ± 0.6	4.11 ± 4.0	5.58 ± 2.0
Right	23.22 ± 4.90	23.00 ± 4.0	19.41 ± 4.0	12.07 ± 1.8	15.85 ± 4.0
% Inhibition Day 21					
Left		29	67	72	62
Right		0.86	16	48	32

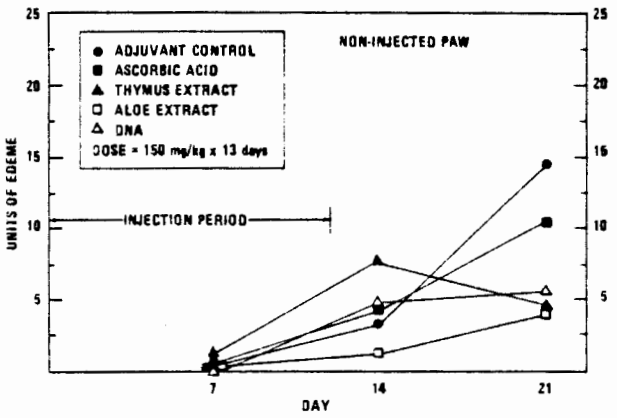

Figure 1. Prevention of adjuvant arthritis with ascorbic acid, thymus extract, aloe extract, and deoxyribonucleic acid. Noninjected paw: immunological effect.

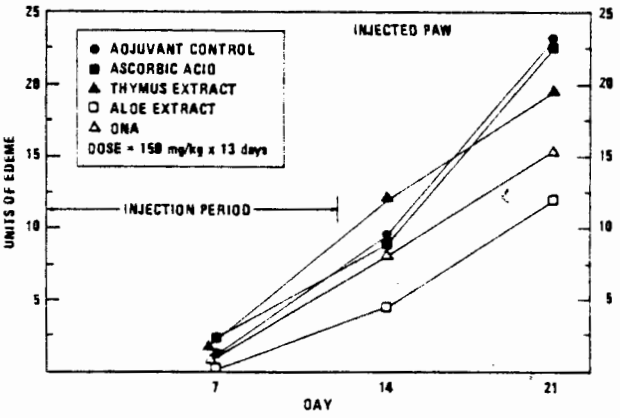

Figure 2. Prevention of adjuvant arthritis with ascorbic acid, thymus extract, aloe extract, and deoxyribonucleic acid. Injected paw: inflammatory effect.

the adrenal cortex, which could serve to hydroxylate corticosteroid hormones.[14] Since ascorbic acid stimulates the production of corticosteroids, which are immunosuppressive to the rat, they could depress the formation of antigen-antibody complexes and the later development of autoantibodies. Also, by depressing collagen breakdown as compared to collagen synthesis, ascorbic acid could depress the immunoglobulin buildup, as well as play a role in reducing the inflammatory process.

Thymus Extract — Prevention

Subcutaneous injections of 150 mg/kg of thymus extract over 13 days produced a 67% inhibition of left "immunological" paw volume in 21 days compared to controls. A 16% inhibition occurred in the right "inflammatory" paw (Table I, Figs. 1 and 2).

We feel that many immunological mechanisms of this autoimmune disease were altered by thymus extract administration. Burnet[25] proposed that developing lymphocyte clones in the thymus are screened out and destroyed if potentially capable of reacting with "self" antigens.

With an injection of foreign antigens into the rat paw, the humoral and cellular limb of the immune response appear to be activated. B and T lymphocytes interact with release of lymphokines causing a local inflammation at the site of inoculation in the right paw. These lymphokines attract and immobilize macrophages and stimulate B lymphocytes to antibody synthesis. The thymus-dependent T lym-

phocytes then cause development of delayed hypersensitivity reactions in the left "immunological" paw.[12]

Failure of screening mechanisms in the thymus results, therefore, in autoimmune disease[25] such as rheumatoid arthritis. Inoculation of *M. butyricum* with oil could alter the "self" antigens and stimulate enlargement of the thymus, with subsequent production of T lymphocytes directed against those altered antigens. Bearing out this concept, thymus enlargement and appearance of germinal centers have been found in many autoimmune diseases, among them systemic lupus erythematosus.[26] Also primary thymomas have been implicated in the development of various systemic disorders, such as autoimmune myositis,[27] systemic lupus erythematosus,[28] and rheumatoid arthritis.[29]

Therefore, the normally nonfunctional adult thymus could be reactivated by antigen-producing thymic hormone once again. Thymic hormone would then induce a state of active recognition within unactivated lymphocytes to this antigen. The foreign antigen altered "self" antigens in the synovium would now be "nonself" and the newly activated lymphocytes would be stimulated to produce antibody for them.

However, administration of thymus extract may inhibit the diseased state by negative feedback on the newly reactivated thymus. Thymus enlargement could decrease and atrophy of germinal centers would occur, resulting in less T cell production and, therefore, less inflammation.

A thymus derived imbalance in pituitary hormones could also result.[29] Since the balance between anti-inflammatory influences (corticotropin, glucocorticoids) and inflammatory (growth hormone, mineralocorticoids) appears to influence antibody production,[30] thymus activity could have an indirect effect on antibody mediated through its effect on the pituitary.

Thus, an increase in thymus extract may shut down the thymus, resulting in an imbalance in pituitary hormones and a resultant shift to the anti-inflammatory influence of corticotropin and glucocorticoids. Lympholysis, decrease in antigen-antibody formation, and resultant decreased inflammation would thereby occur in the synovium of the right "inflammatory" paw. This shift could result in T cell lympholysis, and a subsequent decrease in cell-mediated immunity. Thus, this decrease in delayed hypersensitivity reaction would explain the 67% decrease in left paw volume that we observed over 21 days (Table I, Figs. 1 and 2).

Thymus extract, therefore, could be immunosuppressive because of negative feedback on the thymus and interaction with delicate pituitary balancing mechanisms on other hormones.

Aloe Extract — Prevention

Aloe has antipyretic and anti-inflammatory activity. It provides ascorbic acid when used as a nutrient source.[11-14] Aloe has been utilized for many years as an anti-inflammatory agent in the treatment of burns and skin conditions. In China, Tibet, and India, eczematous skin conditions have been treated with aloe.[15] Collins and Collins[15] reported beneficial effects when freshly split leaves of *Aloe vera* were applied locally to x-ray burns. Rowe and co-workers, when using aloe leaf in x-ray burn treatment, found the curative principle within the pulp and rind of the leaf. Commercial leaves and official aloe preparations did not contain these active ingredients.[16] To avoid this dilemma, fresh aloe leaf extract was injected during the prevention and regression studies.

Aloe significantly reduced inflammation. Average right paw edema within control animals increased from 1.14 ± 0.7 to 23.22 ± 4.9 within a 21-day period compared to aloe increases measuring 0.5 ± 0.7 to 12.07 ± 1.8. A 48% inhibition of inflammation occurred within the right paw because of the subcutaneous injections of 150 mg/kg of aloe extract over 13 days (Table I, Figs. 1 and 2).

Average left paw edema within control animals increased from 0.9 ± 0.42 to 14.69 ± 3.98 within a 21-day period compared to aloe increases measuring 0.43 ± 0.6 to 4.11 ± 4.0. A 72% immunological inhibition occurred. This immunological inhibition is the largest one recorded for the prevention groups (Table I, Figs. 1 and 2).

The biosynthesis and release of prostaglandins from the preoptic hypothalamic area produces fever and inflammation.[37] Zurier[38] found that prostaglandin E_1 inhibited acute carrageenan-induced inflammation and chronic joint inflammation. Also, prostaglandin E_1, when administered to adrenalectonized rats, prevented adjuvant arthritis, reduced the acute inflammatory reaction at the adjuvant injection site, and decreased the amount of circulating small lymphocytes. Therefore, both studies indicate that prostaglandin E_1 is anti-inflammatory in acute and chronic models. Also, its effect is not mediated wholly by stimulation of the adrenal or pituitary glands.[38] So, inhibition of prostaglandin synthesis and release could be the mechanism of action whereby aloe reduces fever and inflammation.

Certain experimental *in vitro* tumors release prostaglandins which, in the presence of bone, may cause lysis of that bone.[39] Prostaglandin concentra-

tions increase in the synovial fluid of rheumatoid joints, and within inflammatory sites. We believe that aloe corrects bone destruction and degradation, because of prostaglandin inhibiton. Xeroradiographs show gross osseous degeneration within prevention controls as compared to changes within the aloe group (Fig. 3).

DNA – Prevention

Subcutaneous injection of 150 mg/kg of DNA over 13 days produced a 62% inhibition of left "immunologic" paw volume in 21 days compared to controls. A 32% inhibition of edema occurred in the right "inflammatory" paw (Table I, Figs. 1 and 2). We feel RNA functions in the immunological process of recognizing and processing foreign antigens. RNA has anti-inflammatory and immunosuppressive activity.[16, 17, 40] RNA produces a strong reduction in gammaglobulins within adjuvant-induced arthritic rats, indicating that the antibodies to antigenic stimulus are not produced.[16] RNA combines with antigen-forming RNA-antigen complexes.[41–43]

Deactivation of the thymus has a critical function in suppressing the immune response. Thymectomy performed on neonate rats weakens the systemic polyarthritis produced by adjuvant inoculation in mature rats.[44, 45] Thymus humoral factor stimulates DNA synthesis and the rate of lymphocytic cell division.[46] Dabrowski[47] found that, in response to antigenic stimulus, thymectomized rats displayed a lower rate of lymphocytic cell division in lymph nodes compared to nonthymectomized rats. Thus, antigen or antigen-RNA complexes may be triggers for thymus reactivation of thymus-dependent T lymphocytes specific to that antigen.

Figure 3. Xeroradiograph comparison of regression of adjuvant arthritis with daily injections of 150 mg/kg of ascorbic acid *versus* aloe extract (days 21–33). Left: ascorbic acid (day 38). Right: aloe extract (day 38).

Autoimmune phenomena result when the host's defenses react against altered antigen derived from tissue breakdown. Tissue destruction caused by lysosomal degradation induces formation of autoantibodies which cause further tissue damage.[48] Human rheumatoid arthritis is also thought to be caused by an autoimmune mechanism.

Thus, inoculation of *M. butyricum* could alter self antigens, so that they were recognized as nonself by the immunological system. Self DNA could produce self RNA which could attach to altered self antigen in the synovium, resulting in activation of T lymphocytes. However, by injection of nonself DNA in the early stages of arthritis development, nonself RNA could be translated within the host, and could compete with the self RNA for attachment to newly injected *M. butyricum*. As a result, it would be removed from the immunostimulatory system. This postulate could indicate the means of curing rheumatoid arthritis. Injections of nonself RNA which are immunosuppressant could also follow the same mechanisms. Less triggering of the immune response would result. It appears that nonself DNA acts as a competitive inhibitor of the immune system.

Regression Study

A 150 mg/kg dosage of ascorbic acid, thymus extract, aloe extract, or DNA was inoculated from day 21 to 33. Measurements on day 21 provided the baseline values from which we measured peak arthritic response. Statistically, they are expressed as zero units of edema.

On day 28, all except aloe extract left hind paw volumes had decreased. Ascorbic acid produced the most significant reduction in edema in the left hind paw. All right hind paws increased in edema with DNA showing the most marked increase (Table II, Fig. 4).

On day 35, ascorbic acid and thymus reduced edema. They exerted their main anti-edema effect on the left immunological paw. Conversely, DNA inhibited edema poorly in both paws, while aloe regressed edema only within the right paw. Aloe markedly increased edema in the left immunological paw (Table II, Figs. 5 and 6).

By day 38, ascorbic acid continued its antiedemic trend in both the left and right paws. The percent of inhibition tabulated from days 21 to 38 for ascorbic acid was 55 and 31% for left and right paws, respectively. At this time, we noticed an antiedemic trend with aloe. There was an inhibition of 26 and 16%, respectively, for the left and right paws.

Table 2. Regression of Adjuvant Arthritis with Ascorbic Acid, Thymus Extract, Aloe Extract, and DNA

	Adjuvant	Ascorbid Acid	Thymus Extract	Aloe Extract	DNA
Treatment (mg/kg × 13) day 21–33		150	150	150	150
Number of rats	11	10	12	12	10
Final body weight (grams)	376 ± 20	349 ± 9	322 ± 12	328 ± 15	333 ± 13
		Edema of hind paws (volume units ± SE)			
Day 21					
Left	14.69 ± 3.98	7.86 ± 3.26	17.72 ± 6.84	12.81 ± 5.08	16.33 ± 6.98
Right	23.22 ± 4.90	26.68 ± 8.67	24.68 ± 11.48	21.67 ± 8.73	41.16 ± 9.19
Day 28					
Left	24.22 ± 5.70	15.31 ± 5.82	20.38 ± 7.78	41.02 ± 7.60	20.63 ± 6.98
Right	26.72 ± 4.70	33.23 ± 9.66	30.51 ± 10.16	32.71 ± 10.39	33.58 ± 12.10
Day 35					
Left	15.81 ± 4.80	4.81 ± 1.70	12.46 ± 5.50	42.14 ± 4.22	49.26 ± 6.99
Right	22.68 ± 4.90	19.28 ± 6.37	20.30 ± 7.58	18.63 ± 6.52	23.23 ± 9.03
Day 38					
Left	14.18 ± 3.60	6.37 ± 2.09	14.40 ± 9.62	10.56 ± 4.57	17.28 ± 7.40
Right	21.00 ± 4.10	14.46 ± 4.85	14.43 ± 7.24	17.71 ± 6.20	39.91 ± 8.77
% Inhibition, Day 38					
Left		55	−2	26	−22
Right		31	31	16	−90
		Relative weight ratio of hind paws			
H.P. Wt./B. Wt.[a]					
Left	1.28	0.85	1.46	1.16	1.13
Right	1.79	1.29	1.83	1.58	1.41
% Inhibition[b]					
Left		34	−14	9	12
Right		28	−2	12	21

Initial body weight, 175–225 g.
[a] H.P. Wt./B. Wt. = Hind paw weight/body weight × 100.
[b] % Difference from adjuvant control.

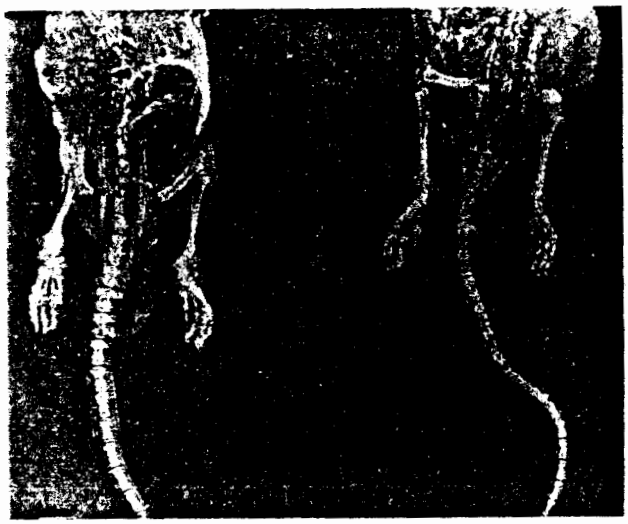

Figure 4. Xeroradiograph comparison of regression of adjuvant arthritis with daily injections of 150 mg/kg of thymus extract *versus* deoxyribonucleic acid (days 21–33). Left: thymus extract (day 38). Right: deoxyribonucleic acid (day 38).

An immense bony lysis and deformation occurred in both paws of the adjuvant control, especially the left immunologic paw. In contrast to ascorbic acid, DNA increased edema in both left and right paws. DNA caused edematous exacerbation of 22 and 90% for the left and right paws, respectively (Table II, Figs. 3–7).

Ascorbic Acid — Regression

Regression inoculations of 150 mg/kg of ascorbic acid were given for 13 days from day 21 to 33, with paw volume measurements taken on days 21 to 38. Significant inhibitions of 55% in the left (immune) paw and 31% in the right (inflammatory) paw resulted compared to the adjuvant control group. These results show that ascorbic acid acts mainly in an immunosuppressive fashion (Table II, Figs. 6 and 7).

Baumgartner et al[49] described three phases of adjuvant arthritis in rats. In the 2-to-4 week phase, splenomegaly and liver dysfunction exist. After 4

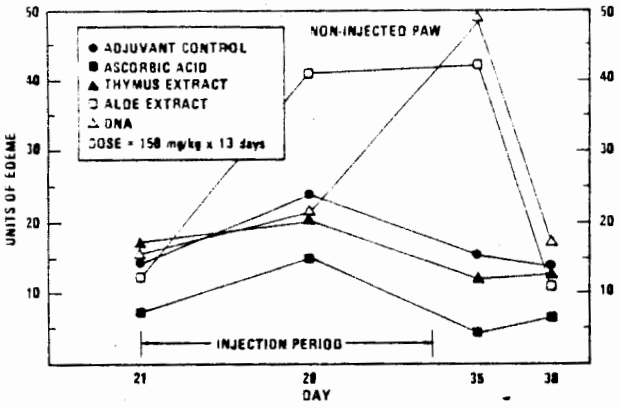

Figure 5. Regression of adjuvant arthritis with ascorbic acid, thymus extract, aloe extract, and deoxyribonucleic acid. Noninjected paw: immunological effect.

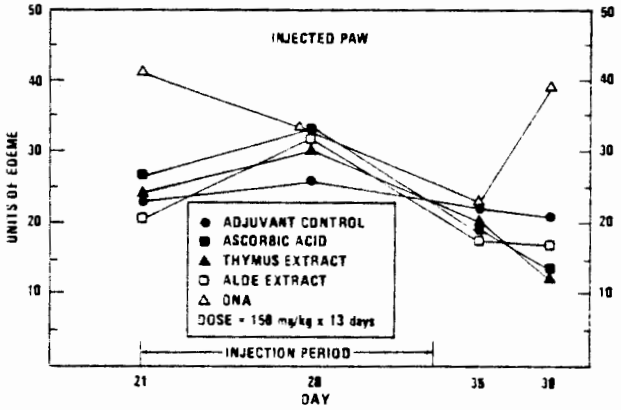

Figure 6. Regression of adjuvant arthritis with ascorbic acid, thymus extract, aloe extract, and deoxyribonucleic acid. Injected paw: inflammatory effect.

weeks, the biochemical abnormalities return to normal, but ankylosis in the extremities and brittleness in the swollen feet is found. Also, extensive osteogenic change with minimal residual inflammation occurs.[49]

Articular cartilage slices switch from forming cartilage collagen (Type II) to formation of skin type collagen (Type I) if lysosomal enzymes are added.[1] Increased lysosomal enzyme activity could thus result in increased arthritic change in cartilage. Chloroquine and prednisone that directly stabilize lysosomal membranes have proven value in treatment of chronic inflammatory disease. Increasing ascorbic acid levels could increase hydroxylation of adrenal corticosteroids. Cortisone formation could increase, causing lysosomal membrane stabilization with resultant immunosuppression. This rationale could explain the substantially decreased osteogenic change in the ascorbic acid-regressed rat on day 38 upon x-ray, compared to the extreme degenerative bone changes in the adjuvant rat (Figs. 3 and 7).

Thymus Extract — Regression

Subcutaneous injections of 150 mg/kg of thymus extract were administered over a 13-day period from day 21 to 33. Edema measured from day 21 to 38 indicated a 2% exacerbation in left paw volume and 31% inhibition in right paw volume compared to controls (Table II, Figs. 5 and 6). Because the disease was already established during the 21 days prior to injection, we feel that immunologically proficient clones of T cells directed toward "self" antigen were already activated. Thus, thymus extract injections would not result in a decrease in left paw volume. By negative feedback, thymus extract probably causes an involution of the thymus just as in the prevention study. However, those T cells that remain immunocompetent would still recognize "self" antigen and cause inflammation in the left paw.

Inflammatory inhibition of the right paw during the regression study was twofold that of the prevention study. The 21-day pretreatment interval allowed significant inflammatory buildup. Upon treatment with thymus extract, we assume that a thymus-mediated influence on pituitary hormones occurred causing a resultant shift to the anti-inflammatory influence of corticotropin and glucocorticoids. This data demonstrates the anti-inflammatory activity of thymus extract and points to the marked benefit of treatment with it during the regression *versus* the prevention situation.

Figure 7. Xeroradiograph comparison of oil control rat injected with 0.1 ml of mineral oil on day 0 *versus* adjuvant control rat injected with 0.1 ml of *M. butyricum* in mineral oil on day 0. Left: oil control (day 38). Right: adjuvant control (day 38).

Aloe — Regression

Subcutaneous injections of 150 mg/kg of aloe extract over 13 days from day 21 to 33 produced an average increase of right paw volume, measured from day 21 to 38, of 21.67 ± 8.73 to 17.71 ± 6.20, as compared to controls with decreases in edema of 23.22 ± 4.9 to 21.0 ± 4.1. The overall inhibition of right paw volume was 16%. Average left paw volume measured from day 21 to 38 decreased from 12.81 ± 5.08 to 10.45 ± 4.57. The overall inhibition of left paw volume was 26% (Table II, Figs. 5 and 6).

Aloe exhibits approximately one-half the regression potential of ascorbic acid. This may be attributed to the presence of ascorbic acid within the aloe. The mechanisms of regression may be the same as ascorbic acid. Aloe is fundamentally effective in prevention, rather than regression, of adjuvant arthritis.

DNA — Regression

Subcutaneous inoculations of 150 mg/kg of DNA were given for 13 days from day 21 to 33. A significant exacerbation of edema occurred. Right "inflammatory" and left "immune" paw edema increased 90 and 22%, respectively, compared to the adjuvant control group. Thus, DNA administration during arthritis regression acts mainly in an immunosupportive fashion (Table II, Figs. 3 and 4).

Since the immune system of the adjuvant arthritic rat has been highly mobilized within a 21-day period, no DNA-mediated competitive inhibition at the joint synovium would occur. Any nonself DNA inoculations from day 21 to 33 could be utilized by the triggered immune system. Thymus humoral factor stimulates DNA synthesis and rate of lymphocytic cell division.[46] Extra DNA could be utilized by a stimulated immune system to produce more T lymphocytes which could react in the synovium with B lymphocytes, forming more inflammation.

DNA and soluble nucleoproteins are found in inflamed joint exudates. Therefore, nuclear antigens from disintegrating granulocytes might complex with antinuclear antibodies to cause articular inflammation.[50] Rheumatoid synovial fluids have been found to contain antibody to soluble nucleoprotein and DNA antigen in 26% of cases compared to 1 of 23 inflammatory nonrheumatoid effusion.[51] Rheumatoid serum rarely contains these antibodies so they are probably locally produced.

Therefore, extra DNA could also be utilized within the synovium to complex with parts of the already triggered anti-DNA antibodies. These complexes might aggregate and thus perpetuate the articular inflammation. Similarly, DNA could be utilized by the host to create more T lymphocytes and thus increase the cell-mediated response in the left paw.

Summary

Prevention of adjuvant arthritis in rats injected with *M. butyricum* was best obtained with 150 mg/kg of aloe extract and least obtained with 150 mg/kg of ascorbic acid. Regression of adjuvant arthritis was best obtained with 150 mg/kg of ascorbic acid. We noticed, however, an increase in the adjuvant arthritis disease state when deoxyribonucleic acid was used in the regression study. These data succeed in exploring future areas of therapeutic substances for the treatment of rheumatoid arthritis and presently provide the podiatrist with effective treatment possibilities.

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