Establishment of behavioral parameters for the evaluation of osteopathic treatment principles in a rat model of arthritis

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Unilateral arthritis was produced in rats by use of methylated bovine serum albumin in a model of antigen-induced arthritis. The progression of arthritis was measured by computerized motion analysis, bilateral joint circumference, voluntary extension force of the hindlegs, and length of ankle extension. Animals with induced arthritis were assigned to treated and untreated groups on the basis of approximately equal deficits by the parameters measured. A third group of rats, which did not have arthritis induced and received no treatment, served to establish mean normal parameters. Modified techniques of muscle energy, passive movement of the ankle and knee, and passive myofascial stretch were applied to the animals, and the animals were exercised in a mechanized exercise wheel. Parameters associated with gait were examined by computerized motion analysis of walking. Animals treated with manipulation and exercise showed significantly relative to untreated animals with antigen-induced arthritis in vertical ankle lift, ankle-based and foot-based stride lengths, knee circumference, and normalized extension of the ankle. The results demonstrate that the parameters identified can be used to detect functional deficits and significant improvement from those deficits can be derived from a nonpharmacologic treatment paradigm that includes osteopathic manipulation and exercise in an animal model of arthritis. These parameters may be useful in the identification of the relative benefits of independent treatment variables including frequency of osteopathic manipulation and exercise and the relative benefits of each in this model. Also, they may elucidate how these treatments produce their beneficial effects clinically.

(Key words: Arthritis, osteopathic manipulative treatment, exercise, rat model, behavioral evaluation)

Up to 5% of Americans younger than 45 years and up to 8% of the older population have some form of arthritis. Arthritis is an inflammation of a joint and its articular cartilage, usually accompanied by pain, swelling, and biomechanical and structural changes. Arthritis exists as two clinically distinguishable disorders: rheumatoid arthritis and osteoarthritis. Rheumatoid arthritis is a systemic disease in which joint inflammation may be only one of several systemic manifestations, whereas osteoarthritis is usually restricted to one or more joints and may include the spinal column. Both rheumatoid arthritis and osteoarthritis involve inflammation and degenerative changes in joints which may be extremely disabling.

Because inflammation of arthritic joints may either produce or participate in permanent degenerative changes in affected joints, primary management of both forms of arthritis typically includes the administration of analgesic and anti-inflammatory agents to reduce pain and slow disease progression. In addition, it is becoming recognized that activity is important in maintaining the function of joints.

From the viewpoint of the osteopathic physician, arthritis produces a cycle of soft tissue changes similar to traumatic insult, and manipulation and exercise are used to break the cycle and maintain or improve joint function. Thus, manipulative techniques used are designed to alleviate responses associated with the disease which lead to disease progression.

The cycle of soft tissue changes associated with arthritis are usually associated with initial pain due to inflammation or trauma. Joint pain causes increases in muscle tension and shortening in the region, increased inflammation and edema, and, eventually, restricted joint motion, limited tendon function, and fascial shortening. The lack of joint motion may further encourage degeneration and fibrosis. Associated increases in sympathetic activity may further encourage degeneration and fibrosis. Associated increases in sympathetic activity may also sensitize the patient’s perception of pain, both peripherally and centrally, and play a role in the muscle spasm and contracture that limit range of motion and increase deformity. Increased sympathetic activity also may compromise blood and lymphatic flow as the result of vasospasm, therefore reducing delivery of nutrients and removal of waste products. The inflammation causes a decrease in diffusion of nutrients and waste products, and decreased blood flow.

The basic rationale of exercise and manipulative treatment is to maintain the range of motion of the affected joints, increase blood flow to periarticular tis-
Joints with rheumatoid arthritis require the articular surface is subjected to compressive force may be found in observed that cartilage is best preserved by repeated injection of the protein in the synovial membrane. In this manner, activity of the joint should increase the exchange of synovial fluid. The exchange of fluid in cartilage also appears to depend on varying pressure loads.

When a load is placed on an articular surface, fluid is readily extruded which lubricates the joint space. In turn, removing a load results in the reabsorption of the fluid by the matrix. The net effect of these two activities is functionally the same as a pumping mechanism for the transport of medications, nutrients, immune complexes, and metabolic wastes through the avascular cartilage by diffusion. Variables affecting this diffusion are blood flow, the concentration of nutrients and waste products in the joint region, and the permeability of the synovial membrane. In this manner, variation in joint loading through activity may be important for maintaining the nutrient status of the joint, particularly in instances of increased metabolism. Joints with rheumatoid arthritis require up to 20 times the normal oxygen consumption, whereas the effective synovial blood flow may be reduced.

In arthritic conditions, it has been observed that cartilage is best preserved where the articular surface is subjected to intermittent compressive contact. This compressive force may be found in manipulation and exercise. The enhancement of fluid exchange within the joint should therefore be a goal of joint manipulation.

Arthritic change is usually manifested first in the minor motions of a joint. Repair is limited to the periphery of the joint, where the cartilage is immediately adjacent to the synovial membrane. In these regions, the lining cells proliferate and produce a form of fibrocartilage rather than normal articular cartilage. If damage occurs outside this region, the cartilage fails to repair because of the inability of mature cartilage to mitose. The limited repair capability of joint articular surfaces emphasizes the importance of minimizing the progression of disease in treatment. Although degenerative changes in arthritic joints cannot be completely halted by any treatment, the interruption of the cycle of the pathologic process should reduce the rate of disease progression.

Animal models of arthritis have been used primarily for testing the potential efficacy of anti-inflammatory agents in joint disease. Joint inflammation is typically produced through the induction of an allergic inflammatory reaction to an antigen that induces a secondary inflammation of joints. In the adjuvant-induced model of rheumatoid arthritis, the general inflammatory reaction is generated through the injection of mycobacterium cell walls in paraffin oil. There are limitations on the type of adjuvant used and the strain of rat. On repeated challenge, the joints of the animal become involved in the inflammatory process. Active disease becomes evident at around 14 days postinduction. Joint involvement is typically monitored by the degree of joint swelling, but degenerative changes simultaneously occur in bone, cartilage, and synovial membranes. The model creates a reliable method of investigating rheumatic diseases, especially for aggressive modes of therapy.

Antigen-induced arthritis is a modification of the adjuvant model in which animals are sensitized to a foreign protein by repeated injection of the protein in an adjuvant mixture. After sensitization, the foreign protein is injected directly into the joint, resulting in a more localized inflammation of a particular joint. The type of adjuvant preparation used in such models will not induce so severe a generalized arthritic response associated with the previously described adjuvant models, but sensitizes the animal to the foreign protein enough to precipitate an immune reaction and inflammation of the joint into which the preparation was injected. In this respect, the model resembles osteoarthritis more than rheumatoid arthritis, although some generalized inflammation affecting all joints may be present.

The objective of our study was to determine if the same model could be used to demonstrate behavioral and biomechanical changes associated with a nonpharmacologic treatment that included osteopathic manipulative medicine (OMM) and moderate exercise. A rat model of arthritis demonstrating a clear responsiveness to nonpharmacologic modes of treatment may offer an effective and efficient method for studying the physiologic and tissue changes associated with the application of osteopathic principles to arthritic conditions.

Materials and methods

All procedures involving animals in this study were approved by the institutional animal care and use committee. Animals (n=26) were divided into three groups, two groups (n=9) in which arthritis was induced, and one group of animals (n=8) in which arthritis was not induced. Of the two groups in which arthritis was induced, one group was treated with OMM and exercise, and one group was not treated. The third group, in which arthritis was not induced, received no treatment and served to establish mean normal parameters. Animals were matched for approximate age and gender.

The induction of unilateral antigen-produced arthritis used was based on a model described by Blackham and Griffiths. In this model, albumin bovine methylated (m-BSA) is injected in a base...
of Freund’s complete adjuvant to cause direct trauma to the joint and an autoimmune-like response. At 18 and 11 days before injection into the joints, 0.5 mL of m-BSA (CalBioChem No. 455451, lot 741993; CalBioChem, La Jolla, Calif) in Freund’s complete adjuvant (Sigma F-5881; Sigma, St Louis, Mo) at a concentration of 1 mg/mL was injected into the base of the tail of each of 18 female Sprague-Dawley rats.

Before arthritis was induced on day 0, both hindlimbs of each rat were measured for the leg extension force, circumference of stifte (knee) and hock (ankle) joints, and distance extension of each ankle. Measurement of leg forces were obtained by holding each rat over an electronic scale and allowing one hindleg to carry the rat’s weight and the animal to push against the scale in a support response. To determine the circumference of joints, a string was wrapped around the joint and then measured in centimeters. For measurements of the extension distance of the ankle, a mark was placed on each lateral tibial condyle with the leg extended after the hindlegs had been shaved. A metric ruler was used to measure the distance between the lateral tibial condyle and the fifth metatarsophalangeal joint.

Animals were anesthetized with ketamine (80 mg/kg of body weight) and xylazine (15 mg/kg). Saline solution, 50 µL, containing 0.5 mg of m-BSA was injected into the right knee joint and right ankle joint of each animal. Sterile saline solution, 50µL, was injected into the left knee joint and left ankle joint capsules, which served as internal controls.

The 18 rats were measured in the three chosen parameters on days 7, 9, 11, and 13 after injection of antigen into the joints. On day 14, the differential (antigen-injected contralateral control) ankle circumference was used to evenly divide these 18 animals into two groups—treated and untreated—on the basis of equal deficits. Such external measurements of joint size are the most frequently used parameters to monitor joint disease in this model. On day 15, a Peak Performance, two-dimensional computerized gait analysis (Englewood, Colo) was done for baseline arthritic changes of the right hindleg (trial 1). Light-reflective tape (3M) markers (3M Retractable Tape Part No. 7610, West Caldwell, NJ) were placed at the crest of the ilium, the lateral tibial condyle, the lateral malleolus, and the metatarsals that correspond with hip, knee, ankle, and foot calculations. At least 12 stride lengths of each animal were videotaped; a biomechanical engineer chose the two most consistent, consecutive strides for computer analysis. An independent biomechanics laboratory did the video processing and analysis.

After the videotaping, modified osteopathic manipulative treatment (OMT) was initiated in the treated group. The nonpharmacologic treatment paradigm was designed to include the basic elements of what would normally constitute a clinical treatment course, but with a frequency thought to be great enough to assure some result in animals, taking into account that animals would not voluntarily contribute to the maintenance of joint flexibility or strength during the treatment period. Therefore, the frequency of OMT and forced exercise was far greater than it is in the clinical setting.

Manipulative treatments were conducted under the direction of an osteopathic physician (T.A.S.) by student physicians with at least 1 year of training in OMM. Treatment consisted of passive range of motion of the right ankle and knee joint and modified muscle energy and passive myofascial stretching of the right hindlimb repeated 10 times each or sustained for 10 seconds, respectively.

Manipulative techniques were modified slightly in order to use the animals’ natural responses to restraint and reflexes to establish limits of motion force. All manipulative treatments were restricted to levels or ranges in which the animals did not demonstrate marked discomfort.

For passive range of motion treatment, one worker restrained the animal while another grasped the animal’s foot and pushed it and the knee into a flexed position. At a point of maximal flexion, the animal would demonstrate a reflex extension from discomfort, and fasciculations of the gluteus muscle could be observed. Other modified muscle energy treatments consisted of inducing the animal to push or pull on the affected leg at maximal extension. This maneuver was accomplished by gently restraining the animal’s forelimbs and body while allowing the animal to push or pull on the affected hindlimb against the investigator’s finger as a potential escape maneuver or pull away from light pressure applied to the foot with maximal extension of the leg. Modified passive myofascial stretching consisted of applying a light pull on the limb at full extension at a point just before the animal would push or pull against the applied force. The pressure was held until a release was detected.

The manipulative treatments were followed by 5 minutes of moderate exercise on a mechanized exercise wheel. Measurements were done 3 days a week on all subjects, whereas the modified manipulative procedures and exercise of the treated group were done 5 days a week. The treated group received a total of 23 sessions of OMT and exercise. A second videotaping (trial 2) of the animals was done on day 43 for posttreatment analysis. Light-reflective tape was again placed on the same anatomic landmarks. The eight untreated nonarthritic rats were analyzed in parallel for control and comparison to show the magnitude of normal variation during the experiment.

Statistical analysis was done by using a Statview 4.1 analysis (Abacus Concepts Inc, Austin, Tex) on a Macintosh computer. Comparisons between pre- and posttreatment groups on motion analysis were made by analysis of variance. Comparisons between groups over time from measurements done continuously over the treatment period (such as the circumference of joints) were made by analysis of variance with repeated measures. A probability value of less than .05 was regarded as being statistically significant.
Results
Motion analysis
The computerized gait analysis parameters used in the two trials were foot- and ankle-based stride length, improvement in vertical ankle and foot lift, and range of motion of the ankle and knee joint (Figure 1). The untreated nonarthritic rats were used to show deficits induced at trial 1 (before treatment) and the amount of improvement or return toward normal of the deficit at trial 2 (after treatment). The vertical lift was determined by the amount of displacement on the X axis on a Cartesian plane exhibited by the lateral malleolus and metatarsophalangeal markers. Range of motion was calculated by the angle created at the joint during the stride. Stride length was derived from the amount of movement of the Y axis.

Before treatment, rats assigned to both treated (P < .026) and untreated (P < .026) groups had significantly shorter foot-based stride lengths than the untreated nonarthritic animals (trial 1) but did not differ from each other (trial 1, P > .9999). At the end of the experiment, the treated group showed a significant increase in foot-based stride length after treatment as compared with the untreated group (trial 2, P < .026) (Figure 2, top) and no longer differed from normal values. The untreated arthritic group showed no change in foot-based stride length with time and had a significantly shorter stride length than the treated group (P < .026) and the untreated nonarthritic group (P < .034) at the conclusion of the treatment period. The ankle-based stride length (Figure 2, bottom) had a slightly higher variability, but a similar pattern. There was significant difference between the animals receiving OMT and the untreated nonarthritic group before treatment (P < .031), but the untreated arthritic group did not show a significant deficit compared with the untreated nonarthritic group (P < .09). However, with treatment, the group receiving OMT increased their stride length so that there was no difference between them and the untreated nonarthritic group in this measurement by the end of the experiment (trial 2, P < .942). During the same period, the rats in the untreated arthritic group showed no change in stride length over time and their stride length was significantly less than that of the group receiving OMT (P < .007) and of the untreated nonarthritic animals (P < .010) at trial 2 (Figure 2, bottom).

The vertical ankle lift measurements (Figure 3) varied appreciably between individual animals. Accordingly, this parameter was analyzed as the amount of improvement between trials (posttreatment [trial 2] and pretreatment [trial 1]). The group receiving OMT showed significantly greater improvement over time (trial 2 from trial 1) compared with the untreated arthritic group (P < .025). The group that had OMT was able to demonstrate an increased lifting of the ankle during a stride after treatment. The improvement in vertical foot lift from pretreatment levels by animals receiving OMT also showed a similar trend to improvement in ankle lift, but this improvement was not statistically significant (data not shown).

In both the treated and untreated groups of rats with antigen-induced arthritis, a significant (P < .018) deficit in ankle range of motion was present at the pretreatment trial compared with the untreated nonarthritic rats (data not shown). There was a trend toward improvement in ankle range of motion for both groups at trial 2, but this improvement was not statistically significant. The knee range of motion showed little difference between manipulated, untreated arthritic, and untreated nonarthritic rats at pretreatment, and this trend continued with posttreatment analysis.

Other measurements
The parameters used to monitor the course of the antigen-induced arthritis during treatment included voluntary extension force of the hindleg, voluntary extension length of the ankle joint, and the circumference of the ankle and knee for both control (saline-injected side opposite to antigen injection) and experimental (antigen-injected) joints. Both the control and the antigen-injected sides showed differences over time in several parameters, suggesting some improvements in all animals with time and some effect of either the control injection or the sensitization procedure.
to the antigen on joint size and function.

Significant differences were observed between the group treated with exercise and the group of OMT-treated and untreated arthritic rats in the distance of voluntary ankle extension and extension force against a balance. Both groups did improve over time, but there was a more rapid improvement in the manipulated group. Normalized ankle extension (Figure 4) of the limb (control, antigen-injected) differed over time by analysis of variance with repeated measurements between manipulated and untreated arthritic animals (P < .027). Both groups reached or exceeded the ankle extension of the control side by the end of the experiment. The ankle extension was significantly better in manipulated animals compared with untreated arthritic animals at 3 weeks (P < .002) and at 4 weeks (P < .02) after treatment. In the final week of treatment, the extension of the treated ankle exceeded the control side of the same animal, a further indication that joints on the control side may have been affected by the sensitization procedure.

The force exerted by the antigen-injected leg differed between treatment groups over time (P < .006; Figure 5). Individual animal improvement from pretreatment values (posttreatment to pretreatment, T2-T1) was significantly greater with OMT at weeks 3 (P < .033) and 4 weeks (P < .035) after initiation of treatment.

Measurements of ankle and knee size differed between manipulated and untreated arthritic groups over time in both the control saline-injected and the antigen-injected joints. This difference was another strong indication that the sensitization may have induced some generalized joint swelling. On the saline-injected (control) side, ankle circumference varied significantly between manipulated and untreated arthritic groups over time by analysis of variance with repeated measurements (P < .042; Figure 6). Specifically, a slow increase in size was observed in the untreated arthritic group whereas the ankle in the exercised and manipulated group decreased slightly (Figure 6). The difference between these groups was significant at 4 weeks after treatment (P < .016) when compared with the pretreatment time. The control knee followed a similar pattern, but was more variable, perhaps because of the inclusion of muscle mass in the measurement. The circumference of the control knee differed significantly between the OMT-exercise-treated and untreated arthritic rats (P < .017) and within groups over time (P < .023) with a smaller circumference in knees in the manipulated and exercised animals (data not shown).
Figure 3. Vertical ankle lift expressed as the difference in average ankle lift between trials 2 and 1. Manipulated group shows significant (P<.025) increased vertical movement of ankle during a stride compared with untreated arthritic group.

Figure 4. Normalized (control antigen-injected) mean ankle extension over time after induction of arthritis. Manipulated group improved faster than untreated arthritic group (P<.027) and differed significantly at third and fourth weeks of treatment. Negative values show extension distances greater than those on contralateral (control) side.

On the antigen-injected side, the circumference of the ankle did not vary significantly between the OMT-exercise-treated and untreated arthritic rats over time (P<.56). However, the circumference of the antigen-injected knee did show a different course of recovery over time between treated and untreated groups (interaction of groups over time; P<.046), with the knees of the manipulated animals being smaller than those of the untreated animals (P<.007) by the 4th week of treatment (Figure 7). The weights of the animals did not change in relation to the increases seen in ankle and knee circumferences.

Discussion

The primary objective of the present study was to identify parameters of measurement through which significant benefits of a nonpharmacologic treatment regimen (OMT and exercise) could be demonstrated in an animal model of arthritic disease. The treatment chosen was similar to the nonpharmacologic approach to the treatment of arthritis used clinically by osteopathic physicians, who recognize the structural and systemic effects of disease. The data demonstrate that such treatment can produce improvements in behavioral parameters, direct measurements, and joint function in an animal model.

Of the parameters measured, those resulting from computerized gait analysis appeared to be some of the most sensitive and reliable. In these measurements, it was possible to demonstrate that treatment significantly increased vertical ankle lift, foot-based stride length, and ankle-based stride length. Trends in other motion analysis parameters followed a similar pattern of better recovery in the manipulated group. The measurements of joint size and behavior-related parameters of voluntary limb extension force and distance were also used to monitor the progress of the treatment and in the final analysis of treatment effectiveness. The treated group had significant improvements in these measurements, including knee circumference, leg extension force, and ankle extension distance, over the untreated arthritic group. These measurements suggest that the treatment did produce increases in the functional "usefulness" of the affected limb in a manner similar to that expected from clinical treatment of joint disease.

The data also showed some differences on the saline-injected control side, in which the size of the joint decreased with time, and significantly with treatment. This effect may suggest that some
generalized inflammation and joint swelling had been induced initially, probably by the allergic response to the adjuvant and through injection damage. The improvement in ankle extension to exceed that of the control side also suggested some deficit in the control side. Because the only treatment afforded to the control side was exercise, the differences between treated and untreated arthritic groups on the control side suggest that exercise alone may have promoted the reduction of inflammation in affected joints. Although the swelling of the ankle joint appeared very reliable, alterations in the circumferences of the knees in animals were complicated by the fact that some muscle mass was included in this measurement. Muscle wasting with decreased use in severely affected animals as well as hypertrophy of the control side may complicate the interpretation of data in this measure, although significant changes with treatment were observed. A more complete monitoring of joints during the induction process will be necessary to demonstrate a generalized joint swelling in this model.

Considerable variability existed in the severity of the antigen-induced arthritis within groups, although animal groups were matched for severity of the induced arthritis. Measurements of external joint size of the ankle were used to divide the animals into the two treatment groups. It may be desirable in future studies to subdivide animals into groups of varying severity to identify possible limits on the usefulness of OMT and exercise, particularly in severe joint disease. The numbers of animals used in the present study did not allow such subdivisions in the statistical analysis. External joint size did, however, prove to be an effective means for initial group subdivision.

The behavioral parameters chosen in this study allowed for an objective analysis of the combined effects of OMT and exercise on the arthritic joints. From the present data, it is not possible to determine the relative benefits of the exercise, OMT, or an interaction between the two. Exercise has been shown to decrease disease activity in patients with rheumatoid arthritis and patients with osteoarthritis. Because of the benefits they confer, techniques of range of motion, stretching, strengthening, and aerobic exercise in preventing deconditioning and contractures are currently gaining favor in the medical community. The exact mechanisms by which these modes of therapy improve the arthritic joint are unclear.

**Comment**

The animal model of arthritis described should prove useful in defining anatomi-
ic and physiologic changes associated with the benefits of treating arthritis according to osteopathic principles. The model used does produce degenerative changes in joint surfaces\(^1\) that can be quantified. Preliminary analysis suggests that degenerative changes in affected joints were present in both knee and ankle joints of the affected side, but quantitative comparison of manipulated and untreated joints is still in progress.

References


