Pulsed Radio Frequency Therapy of Experimentally Induced Arthritis in Ponies

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ABSTRACT

The effect of pulsed radio frequency therapy (PRFT) was evaluated on seven ponies with no arthritis and in 28 ponies in which arthritis was created using intra-articular amphotericin B to induce synovitis in the right middle carpal joint. The ponies were divided into five treatment and two control groups. Two levels of arthritis were created and two dosage levels of PRFT were evaluated. The effect of PRFT on arthritic and nonarthritic joints was measured by comparing synovial fluid parameters, the degree and duration of lameness, the range of carpal motion, and carpus circumference, for treated and untreated groups. Lesions seen radiographically, at gross pathology, and by histopathology were also compared between the treated and control groups. In the ponies with a mild form of induced arthritis, PRFT significantly ($p < 0.05$) reduced the severity and duration of lameness, swelling of the carpus, and the severity of gross pathological and radiographic changes. In these ponies the synovial acid phosphatase levels were lower, the mucin clot quality was superior, and the synovial protein levels were lower for the ponies receiving PRFT as compared to the arthritic ponies receiving no treatment. A dose response effect was evident. In ponies with a slightly more severe form of arthritis, PRFT was evaluated at one dosage level. The treated ponies were significantly improved over the untreated ponies with respect to carpal range of motion, degree of lameness, carpus swelling, and radiographic lesions. No deleterious effects were noted when normal, PRFT treated, middle carpal joints were compared to contralateral untreated, normal joints. It was concluded that significant beneficial effects resulted when affected ponies were treated with PRFT.

RÉSUMÉ

Les effets sur les structures articulaires reliés à l'application d'ondes de fréquence radio (pulsed radio frequency therapy, PRFT) ont été évalués chez sept poneys dépourvus d'arthrite et chez 28 poneys atteints d'arthrite expérimentalement induite par l'administration d'amphotéricine B dans l'articulation inter-carpienne droite. Les poneys ont été divisés en cinq groupes expérimentaux et deux groupes témoins. Les effets articulaires des PRFT ont été évalués en comparant les liquides synoviaux, le degré et la sévérité de la boiterie, la flexibilité du carpe, et la circonférence du carpe des animaux traités au PRFT à ceux des animaux non-traités. Les lésions observées en radiologie, et celles observées lors des examens macroscopiques et histopathologiques ont aussi été comparées. Chez les poneys présentant une arthrite légère, PRFT a réduit de façon significative ($p < 0.05$) la sévérité et la durée de la boiterie, l'enflure du carpe et la sévérité des lésions pathologiques macroscopiques et des lésions radiologiques. Chez ces poneys, le taux de phosphatase acide dans le liquide synovial était inférieur, la qualité du caillot de mucine était supérieure, et le taux de protéines du liquide synovial étaient inférieurs chez les poneys traités au PRFT comparativement à ceux n'ayant pas reçu de traitement. Une association dose-réponse était évidente. Chez les poneys ayant une arthrite légèrement plus sévère, seulement un niveau de PRFT a été évalué. Les poneys traités, comparés aux non-traités, présentaient une amélioration significative de la flexibilité du carpe, du degré de boiterie, de l'enflure du carpe et des lésions radiologiques. En comparant les articulations carpiennes normales traitées au PRFT et celles non-traitées, aucun effet néfaste n'a été noté. Les auteurs concluent que des effets bénéfiques significatifs ont été associés au PRFT compte tenu du modèle expérimental utilisé. *(Traduit par Dr Jean-Pierre Lavoie)*

INTRODUCTION

Degenerative joint disease (DJD) is a serious problem in equine athletes (1). In these horses, an untreated inflamed joint will progressively deteriorate through a cascade of degenerative changes in the cartilage,

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bone and soft tissue (1). As DJD progresses, the potential for an affected horse returning to soundness diminishes (2). Researchers continue to attempt to identify medications which will arrest the progression of the disease (3-11). Most approaches to managing horses with DJD include reduction or alteration of the training and working program, changes in shoeing, physiotherapy and medication (12). Drugs useful for the management of pain and/or inflammation in humans with arthritis are used in horses with DJD to allow them to continue as athletes.

Preliminary experiments on rats with arthritis induced by an adjuvant of heat killed *Mycobacterium tuberculosis* indicated that a beneficial effect was obtained when pulsed radio frequency therapy (PRFT) was applied to the affected joints (Sciubba JJ, unpublished report). Treatment resulted in an improvement (based on a clinical scoring system) when treated groups were compared with untreated groups. However, a consistent difference could not be shown between treated and untreated limbs due to the effect of energy scatter from the PRFT apparatus.

The objectives of this study were to determine if PRFT:

1. Reduced the signs of clinical lameness in ponies with experimentally induced arthritis.
2. Inhibited the pathological processes associated with experimentally induced arthritis in ponies.
3. Created signs of clinical lameness in ponies that were sound prior to undergoing treatment.
4. Induced pathological lesions in normal joints.
5. Had a dose related effect on clinical lameness in ponies.
6. Had a dose related effect on the presence of pathological lesions associated with experimentally induced arthritis in ponies.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Following purchase, 40 mature ponies weighing between 137 and 268 kg were maintained in isolated, group housing for a 3 wk adaptation period.

TABLE I. Summary of group treatments

Group	Number of ponies	Treatments: Left middle carpal joint	Treatments: Right middle carpal joint	PRFT[a] right carpus
A_1T_0	10	arthrocentesis	Amphotericin B day 1	no treatment
A_0T_1	4	arthrocentesis	arthrocentesis day 1	level 1[b]
A_0T_2	4	arthrocentesis	arthrocentesis day 1	level 2[c]
A_1T_1	5	arthrocentesis	Amphotericin B day 1	level 1
A_1T_2	5	arthrocentesis	Amphotericin B day 1	level 2
A_2T_0	5	arthrocentesis	Amphotericin B days 1, 4, 18	no treatment
A_2T_2	5	arthrocentesis	Amphotericin B days 1, 4, 18	level 2

[a]PRFT = pulsed radio frequency therapy
[b]Level 1 = PRFT applied to the dorsal and palmar surfaces of the carpus. Each surface received two 11 s bursts of pulsed radio frequency energy delivering 7.7 Joules per burst. Each burst was separated by a 15 s interval
[c]Level 2 = PRFT applied to the dorsal and palmar surfaces of the carpus. Each surface received four 11 s bursts of pulsed radio frequency energy delivering 7.7 Joules per burst. Each burst was separated by a 15 s interval

A standard animal health protocol for experimental animals which is analogous to the "Guide for the Care and Use of Experimental Animals," of the Canadian Council on Animal Care was followed. The ponies were taught to lead at the walk and trot, and trained to trot in both directions on an equine "hot-walker". One week prior to the start of the experiment, the hair coat of both forelegs on every pony was removed from the mid-carpus to the mid-radius by clipping, with a #40 blade. Physical examinations during the adaptation period revealed that two ponies were lame and not suitable for the study. The remaining ponies were numbered and randomly assigned to one of the seven experimental groups (Table I). The A_1T_0 group contained ten ponies, because five ponies which were injected in the right middle carpal joint (MCJ) with amphotericin B and were originally in a group assigned to PRFT treatment to be given 24 h postinjection, could not receive treatment due to a mechanical failure of the equipment. These ponies were reassigned to the A_1T_0 group.

Prior to the start of the experiment, it was determined that any ponies with a nonweight bearing lameness that persisted for three days, or ponies that became recumbent due to lameness, would be euthanized.

EXPERIMENTAL PROCEDURES, OBSERVATIONS AND SCORING OF OBSERVATIONS

The experimental period was 8 wk. Table II summarizes the schedule of

TABLE II. Schedule of sample collections and observations

Weeks post-injection	Radiographs	Synovial fluid exam	Lameness examination	Range of motion	Joint circumference
1		X	X	X	X
2	X		X		X
3		X	X	+	X
4			X	*	X
5	X	X	X	+	X
6			X	*	X
7		X	X	+	X
8	X		X	*	X

X = All groups
+ = Groups A_1T_0, A_0T_1, A_0T_2, A_1T_1, and A_1T_2
* = Groups A_2T_0 and A_2T_2

TABLE III. Observation scoring systems

Lameness evaluation
1 = no evidence of lameness
2 = lameness barely detectable by expert observer
3 = lameness easily detectable, pony has a noticeable head nod
4 = severe lameness, characterized by a marked head nod, pony bearing weight on affected limb
5 = severe lameness characterized by nonweight bearing on affected limb

Radiographic evaluation
0 = normal
1 = synovial effusion and/or mild joint distension
2 = marked joint distension with no bone or cartilage change
3 = minimal bone changes, osteophytes < 2 mm, with joint distension, no evidence of bone lysis or cartilage loss
4 = severe bone changes, osteophytes > 2 mm, with or without evidence of cartilage loss and/or bone lysis

Mucin clot
1 = poor
2 = fair
3 = good

Gross pathology evaluation
A. *appearance of synovial tissue*
0 = normal
1 = mottled reddening
2 = reddening plus proliferative changes
3 = diffuse, dark reddening

B. *appearance of fibrous joint capsule*
0 = normal
1 = mottled reddening
2 = reddening plus proliferative changes
3 = diffuse, dark reddening

C. *appearance of articular cartilage and bone*
0 = normal
1 = discoloration and/or "ground glass" appearance
2 = cartilage loss
3 = cartilage loss plus osteophytes and/or marginal lipping

Histopathology evaluation
A. *synovial membranes*
0 = normal
1 = mild edema and localized clusters of inflammatory cells
2 = slight inflammation and hypervascularity, may include mild localized fusion of synovial villi
3 = mild fusion and fibrosis of synovial villi, may include minimal inflammatory cell infiltration
4 = severe fusion and fibrosis of synovial villi with inflammatory cells present

B. *synovial fibrous tissue*
0 = normal
1 = mild inflammation and mild fibrosis
2 = moderate thickening with mild fibrosis
3 = moderate thickening and moderate inflammatory cell response
4 = moderate to severe thickening with intense inflammatory cellular response and granulation response

C. *articular cartilage and bone*
0 = normal
1 = chondromalacia and/or loss of cartilage matrix architecture
2 = chondromalacia and fibrous replacement of cartilage
3 = osteophyte formation
4 = osteophyte formation with cartilage loss and thinning

sampling and observations that were conducted during the study. In order to have consistent time intervals between samplings or observations, these procedures were conducted on the same experimental day for each group. Table III describes the scoring systems that were used for each observation.

Arthritis induction — A previously described method for inducing arthritis in ponies was used in this study (13). Sterile procedures were followed in performing all arthrocenteses. Synovitis was induced in the right MCJs of the ponies in the A_1T_0, A_1T_1, and A_1T_2 groups by injecting 10 mg of amphotericin B (Fungizone intravenous, E.R. Squibb and Sons, Inc., Princeton, New Jersey) into that joint on day 1 of the experiment. In a departure from the previously described arthritis model (13), the ponies in the A_2T_0 and A_2T_2 groups received 10 mg of amphotericin B in the right MCJs on days 1, 4, and 18 of the experiment. In all ponies the left carpus served as the normal control joint while the right carpus served as the experimental joint. In order that the inflammatory effect of the arthrocentesis be present in both joints, 2 mL of synovial fluid were obtained from the control MCJ when synovial fluid sampling and amphotericin B injections were administered to the right MCJs of the ponies in the A_1T_0, A_1T_1, A_1T_2, A_2T_0 and A_2T_2 groups. In the A_0T_1 and A_0T_2 groups, synovial fluid aspirates were obtained from the MCJs on day 1 of the experiment.

Experimental treatment — The A_1T_0 and A_2T_0 groups received no treatment. The right MCJs of ponies in the other groups received PRFT (Vet-Sonotron, Vet-Sonotron Systems, Inc., Northvale, New Jersey) at two different dosage levels as indicated in Table I. Treatments were administered once daily, from day 2 to day 30. Level 1 treatment provided the dorsal and palmar surfaces of the carpus with two treatment exposures each. Each exposure consisted of an 11 s burst of pulsed radio frequency energy supplying 7.7 Joules (1 Joule = 1 watt-second) in each burst. Each burst was separated by a 15 s time interval. Level 2 treatment

exposed the dorsal and palmar surfaces of the carpus to four 11 s, 7.7 Joule bursts on each surface, with a 15 s interval between each burst.

The A_1T_0 group served as an untreated control group with induced arthritis for comparison with the A_1T_1 and A_1T_2 groups. The A_2T_0 group was the untreated control group with arthritis induced by three injections of amphotericin B which was compared with the A_2T_2 group. The A_0T_1 and A_0T_2 groups were used to determine if either level of PRFT caused changes and/or lameness to the normal joint. In order to use a minimum number of ponies, the left MCJs, which were not exposed to PRFT, served as the controls for the animals in the last two groups.

Lameness evaluation — Throughout the experiment, all clinical lameness evaluations were done by one individual (WHC) who did not work with the ponies on a daily basis, and who was unaware of the treatment group to which the ponies were assigned. Lameness evaluations were conducted on a flat, firm grass surface. Each pony was trotted in a straight line in both directions with the evaluator observing the ponies from each side. A lameness grade, based on the scoring system in Table III, was assigned to each pony for each evaluation.

On day 0 of the experiment, each pony was evaluated for lameness and assigned a lameness score. Subsequent lameness evaluations were done twice weekly for the first 2 wk and then once a wk for the A_1T_0, A_0T_1, A_0T_2, A_1T_1 and A_1T_2 groups. In the A_2T_0 and A_2T_2 groups lameness evaluations were conducted on a twice weekly basis for 4 wk and then once a week for the remaining 4 wk of the study.

Joint circumference — The circumference of the right and left carpi were measured once a week, at the level of the MCJ. To assure consistent placement of the measuring tape, with the pony bearing weight on the leg, the most distal portion of the accessory carpal bone was used as a landmark to locate the tape. Measurements were conducted as the first procedure of the day in order that edema reduction resulting from exercise would not affect the relative joint circumference measurements between the groups.

Synovial fluid samples — Two mL synovial fluid samples were collected in ethylenediaminetetraacetate (EDTA) tubes from the right and left MCJ on day 1, and every other week thereafter. In the joints receiving amphotericin B, a single arthrocentesis was conducted to extract the synovial fluid and administer the drug. One mL of each sample was used for determinations of total white well count, total erythrocyte count, total protein and mucin clot characteristics. The remainder of the sample was stored at -20°C for subsequent determination of acid phosphatase levels (14).

Carpal range of motion — On both forelegs of each pony, Velcro (Velcro USA Inc., Manchester, New Hampshire) hook patches, 1.5 cm square, were secured to the skin on the lateral surface of the leg using gelled cyanoacrylate adhesive (Duro Quick Gel™, Locite Corporation, Cleveland, Ohio). The patches were located at the level of the lateral tuberosity of the proximal radius, over the lateral tuberosity of the distal radius, and over the lateral tubercle of the distal metacarpus. These patches served to retain a reflective band and to maintain repeatable index locations on the legs for serial studies. The reflective bands were 1.2 cm wide and constructed with an inner layer of Velcro hook and pile material and an outer layer of white reflective film (Scotchlite, The 3M Comp., St. Paul, Minnesota). The bands were positioned over the index patches on the leg to be studied, and closed with enough tension to retain their position, but loose enough not to restrict circulation or freedom of movement (Fig. 1a).

The ponies were filmed at a normal trot (1.3 m per s) by a video camera (Panasonic Industrial AG-160 VHS/HQ Camcorder, Panasonic division of Matsushita Electronics Corporation of America, Secaucus, New Jersey) recording at a rate of 60 fields per s, with 350 lines per inch resolution and 1/1000 s shutter speed, mounted on the central, rotating shaft of the hot walker with the center line of the lens 38 cm above the trotting surface. Motion data were collected for the right leg with the pony trotting clockwise on the walker, and for the left leg data the pony was trotted counterclockwise. An on-camera character generator was used to record

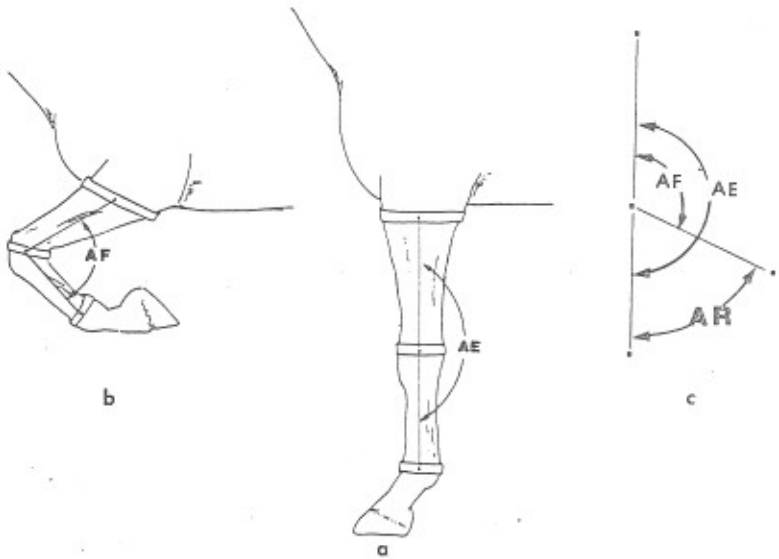

Fig. 1. Diagrams showing the location of reflective tape placed on the forelimbs, and the angles used to determine the carpal range of motion.

on each frame the pony identification number, the date and the day of the study.

The carpal range of motion analysis system used a personal computer (Amiga 1000 computer, Comodore Business Machines Inc., West Chester, Pennsylvania), a high resolution monitor (Sony Trinitron model KZ-1311 CR, Sony Corp, New York, New York), and video recorder (Panasonic Industrial Video Recorder, AG-1950 VHS/HQ, Panasonic division of Matsushita Electronics Corp of America, Secaucus, New Jersey) using the jog-shuttle mode for data frame selection. An interface (Amiga 1300 Genlock Interface, Comodore Business Machines Inc., West Chester, Pennsylvania) was used to superimpose the data frame to be analyzed with the graphics cursor of the computer on the monitor. The maximum extended angle of the carpus, AE (Fig. 1a) was the first to be determined. The cranial and caudal margins of each reflective band were marked with the graphics cursor. The computer program then calculated a midline point for each of the reflective segments and computed the angle defined by these three points (Fig. 1a) The video tape was then advanced frame by frame until the leg was maximally flexed, at which point the angle AF (Fig. 1b) was determined. The angle AR, representing the range of motion, was calculated by subtracting AF from AE (Fig. 1c). Six strides were analyzed, and the average and standard deviation for the data set were calculated.

Radiographs — Cranial to caudal, and lateral to medial view radiographs were made of each carpus for each pony, on experimental days 0, 15, 35, and 56. Identification markings on the films were covered and radiographs for each joint were randomly presented to the evaluator (WHC) who graded them based on the scoring system in Table III.

Necropsy — All the ponies were euthanized on experimental day 57 with a lethal intravenous injection of barbiturate (Beuthanasia-D Special, Schering Corp., Kenilworth, New Jersey). Both carpi were collected from each pony and tagged to identify the limb and the pony. The identification numbers were covered and each carpus was examined and assigned a score (Table III) for the gross pathalogical changes. Sections of fibrous and synovial joint capsule were taken from the dorsomedial surface of the MCJ, and a segment of bone with the overlying articular cartilage was harvested from the articular surface and dorsal margin of the third carpal bone. The harvested soft tissue samples were placed in Bouin's fixative solution (15) for 4 to 6 h and then sectioned at 5 μm and stained with hematoxylin and eosin. Following 72 h in a decalcifying solution (Surgipath Decal 2, D-2128, Surgipath Medical Industries Inc., Greyslake, Illinois), the osteochondral samples were also sectioned at 5 μm and stained with hematoxylin and eosin. The same pathologist evaluated the histological sections and assigned a score to each section, based on the grading system in Table III. Scores from gross pathology categories a, b and c (Table III) were added for each specimen to obtain a gross pathology total score. Scores from histopathology categories a, b and c (Table III) were added for each specimen to obtain a total histopathology score.

DATA MANAGEMENT, MANIPULATION AND COMPARISONS

Numerical measurement or scored observations were tabulated for the following parameters: synovial fluid white blood cell count, synovial fluid protein concentration, synovial fluid mucin clot quality, synovial fluid acid phosphatase concentration, carpus circumference, lameness, right carpus range of motion, MCJ radiographic evaluation, gross pathological evaluation of the MCJs, and histopathological evaluation of the MCJ.

The numerical or score data were manipulated using the formula:

$$Y_n = (R_n - R_0) - (L_n - L_0)$$

where:

Y_n = the net data value for day n.
R_n = the data value for the right leg on day n.
R_0 = the data value for the right leg on day 0.
L_n = the data value for the left leg on day n.
L_0 = the data value for the left leg on day 0.
n = the day of collection or observation and varies from 0 to 56.

To evaluate the change in a parameter and the duration of the change, a test parameter-duration curve was constructed by plotting the net data values on the vertical axis versus the experimental day of data collection on the horizontal axis (Fig. 2). For each pony, the area under the line graph for each parameter was calculated and recorded. Group means and standard deviations for the areas under the test parameter-duration curves were calculated for each parameter.

For each test parameter, the mean areas of the treatment groups were compared to the control group mean area using a simple, one-tailed, *t*-test (16). Significant differences were assumed to occur when $p < 0.05$. In the A_0T_1 and A_0T_2 groups (levels 1 and 2 of PRFT respectively, but no synovitis induction). Areas under test parameter-duration curves constructed for the treated (right) leg were compared to analogous areas derived from the data on the untreated (left) leg using a paired *t*-test (16). The level of significance selected was again $p < 0.05$.

The duration of lameness was the time interval, in weeks, between the onset of lameness and the return of the pony to soundness. The mean duration of lameness was calculated for each group, and the experimental groups were compared to their control groups using a simple *t*-test with significant differences indicated when $p < 0.05$.

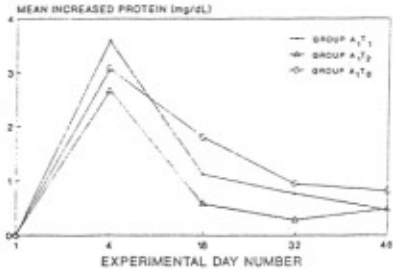

Fig. 2. Test parameter-duration curves showing the mean changes in synovial protein levels within the right middle carpal joints. The areas under each curve were used to compare the experimental groups to the control group.

TABLE IV. Comparison of areas under the test parameter curves, and test parameter scores, for groups receiving 10 mg of intra-articular amphotericin B on day 1

Test parameter	Group A_1T_0 (n = 8)	Group A_1T_1 (n = 5)	Group A_1T_2 (n = 5)	Significance levels A_1T_1 vs A_1T_0 p <	A_1T_2 vs A_1T_0 p <	A_1T_1 vs A_1T_2 p <
Synovial protein (mg/dL)[a]	67.71 ± 47.24	59.56 ± 22.77	36.39 ± 18.27	ns	0.025	0.01
Synovial white cells (cells/μL)[a]	33581 ± 38107	29210 ± 21418	29670 ± 7554	ns	ns	ns
Mucin clot (score)[a]	-40.06 ± 29.35	36.40 ± 12.91	-36.30 ± 21.86	0.005	ns	0.005
Carpal circumference (cm)[a]	36.40 ± 38.93	41.03 ± 29.68	32.87 ± 29.74	ns	ns	ns
Lameness (score)[a]	17.44 ± 12.29	9.00 ± 8.63	17.00 ± 18.59	0.025	ns	ns
Radiology changes (score)[a]	41.44 ± 31.81	23.80 ± 16.91	33.40 ± 18.71	0.05	ns	ns
Reduced range of motion (degrees)[a]	-187.02 ± 269.44	-75.52 ± 132.24	-23.77 ± 277.49	ns	0.05	ns
Acid phosphatase (Sigma units)[a]	159.54 ± 154.63	19.71 ± 55.75	73.91 ± 155.57	0.005	ns	ns
Gross pathology changes (score)[b]	1.53 ± 1.39	0.60 ± 1.14	1.20 ± 0.84	0.025	ns	ns
Histopathology changes (score)[b]	1.25 ± 3.06	1.80 ± 2.59	0.80 ± 1.64	ns	ns	ns
Duration of lameness (wk)[b]	3.38 ± 1.85	1.20 ± 0.84	2.60 ± 2.41	0.005	ns	0.05

[a]The mean ± standard deviation shown are for areas under the test parameter-experimental days curve. Units of area are: Units of test parameter x days
[b]The mean ± standard deviation shown are for the mean score or duration. There is no area under the parameter curve
Group A_1T_0: Controls; no PRFT administered
Group A_1T_1: Received 1 PRFT on right carpus
Group A_1T_2: Received 2 PRFT on right carpus

RESULTS

Three ponies were removed from the study. Two of the ponies from the A_1T_0 group had an arthritis that varied from the amphotericin B model. Pony #8 in the A_1T_0 group developed septic arthritis of the left carpus. She became severely lame (grade 5) and was euthanized. Data from pony #9 of the A_1T_0 group were discarded because a hair shaft, which had elicited a foreign body response, was found within the left MCJ at necropsy. On the day 0 lameness examination, pony #4 in the A_0T_2 group was diagnosed with bilateral degenerative joint disease in the proximal interphalangeal joints of the forelimbs. Due to the unavailability of conditioned ponies, she was not replaced in the group. All lameness that occurred in the remaining ponies was in the right forelimb.

Every pony that had amphotericin B injected in the MCJ became lame in the right forelimb within 6 h of the injection. However, with the exception of pony #8 in the A_1T_0 group, none of the ponies became recumbent or had a persistent grade 5 lameness. The mean duration of lameness of each treatment group are reported in Tables IV and V, and illustrated in Fig. 3.

The ponies did not object to treatment with the Sonotron apparatus. Most of them were initially frightened by the sound emitted from the equipment but adapted to the noise within two or three treatments so that forceful restraint was unnecessary. There was no evidence of skin irritation at the site of treatment on any pony.

In the A_2T_0 and A_2T_2 groups of ponies (three injections of amphotericin B), lameness was much more severe than in the A_1T_0, A_1T_1 and A_1T_2 groups. Video taping scheduled for day 15 was not conducted in these groups due to the severity of lameness

TABLE V. Comparison of areas under the test parameter curves, and test parameter scores, for groups receiving 10 mg of intra-articular amphotericin B on days 1, 4 and 18

Test parameter	Group A_2T_0 (n = 5)	Group A_2T_2 (n = 5)	Significance levels A_2T_2 vs A_2T_0 p <
Synovial protein (mg/dL)[a]	95.16 ± 49.61	112.47 ± 44.7	ns
Synovial white cells (cells/μL)[a]	82650 ± 48115	72440 ± 45595	ns
Mucin clot (score)[a]	-59.00 ± 14.00	-59.00 ± 8.58	ns
Carpal circumference (cm)[a]	127.32 ± 21.95	95.75 ± 36.57	0.01
Lameness (score)[a]	104.00 ± 18.66	74.50 ± 19.84	0.005
Radiology changes (score)[a]	119.40 ± 24.64	103.60 ± 21.99	0.05
Reduced range of motion (degrees)[a]	-530.47 ± 349.52	-182.59 ± 161.29	0.005
Gross pathology changes (score)[b]	4.80 ± 2.17	4.00 ± 1.23	ns
Histopathology changes (score)[b]	6.00 ± 2.55	6.60 ± 2.30	ns
Duration of lameness (wk)[b]	7.20 ± 0.45	6.4 ± 1.82	ns

[a]The mean ± standard deviation shown are for areas under the test parameter-experimental days curve. Units of area are: Units of test parameter x days
[b]The mean ± standard deviation shown are for the mean score or duration. There is no area under the parameter curve
Group A_2T_0: Controls; no PRFT administered
Group A_2T_2: Received 2 PRFT on right carpus

in all the ponies following the second amphotericin B injection. Synovial fluid samples from the A_2T_0 and A_2T_2 group ponies were difficult to obtain and as a result were consistently contaminated with varying amounts of blood. The analysis for acid phosphatase was discontinued in these groups because of the increased levels of this enzyme present in erythrocytes.

There were significant differences between the A_1T_0 and A_2T_0 groups in the gross pathological and histopathological changes seen in the MCJs. The changes seen in the A_1T_0 group ponies were mainly confined to the synovial tissues and the fibrous joint capsule. In the ponies of the A_2T_0 group, in addition to synovitis and capsulitis, degenerative changes were present in the cartilage and bone.

In the total number of strides (1056) analyzed for carpal range of motion, the mean standard deviation was $4.5 \pm 1.1\%$ of the mean angle of carpal flexion. The range of motion increased in both carpi of the A_0T_1 and A_0T_2 group ponies, and in the left carpus in 50% of the A_1T_1 and A_1T_2 group ponies. This incerase was interpreted to be the result of the ponies learning to lengthen their stride as they became more accustomed to trotting on the hot walker. Therefore, scores for left carpal flexion were not subtracted from right carpus flexion scores.

Summarized data and comparisons between the group means for the ponies receiving 10 mg of amphotericin B on day 1 are presented in Table IV. In Table V, summarized data and comparisons between the group means for the ponies receiving 10 mg of amphotericin B on days 1, 4 and 18 are shown. These tables display the group mean values of the area under each test parameter-duration curve. In the case of gross necropsy changes, histopathological changes, and duration of lameness, test parameter-duration curves could not be constructed, and the mean scores of the groups for these parameters are given.

Compared to the untreated control (A_1T_0) group, ponies in the level 1 PRFT (A_1T_1) group had significantly lower areas under the test parameter-duration curves for synovial acid phosphatase, lameness, and radiographic changes. The mean area under the mucin clot quality curve was significantly greater for the A_1T_1 group ponies than for the ponies from both the A_1T_0 and A_1T_2 groups, indicating that during the experimental period, ponies receiving level 1 PRFT had a better quality mucin clot than the ponies in the level 2 PRFT or untreated control groups. The mean duration of lameness was significantly less for the ponies of the A_1T_1 group than for those in the A_1T_0 group and the A_1T_2 group (Fig. 3). The severity of gross pathological changes was significantly less in ponies from A_1T_1 group than in those from the A_1T_0 group.

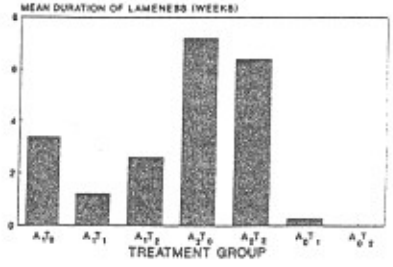

Fig. 3. The mean duration of lameness for each group of ponies is shown. The severity of lameness is not indicated in this measurement.

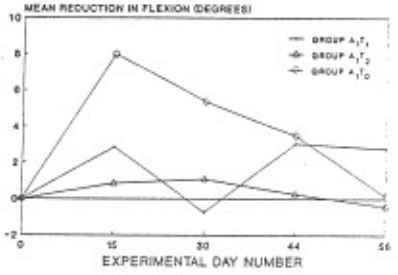

Fig. 4. Test parameter-duration curves showing the mean reduction in carpal range of motion that occurred in the ponies receiving amphotericin B on experimental day 1. Negative values indicate an increase in mean range of motion.

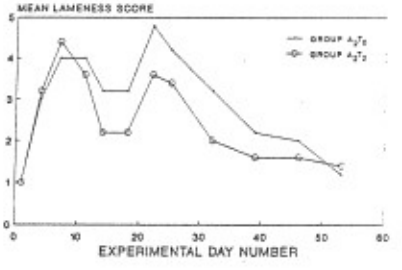

Fig. 5. Test parameter-duration curves showing the mean lameness scores recorded by the ponies receiving amphotericin B on experimental days 1, 4 and 18.

The A_1T_2 group ponies had significantly less area under the mean range of motion reduction-duration curve than the A_1T_0 group ponies (Fig. 4). This indicates that for the duration of the experiment, there was less reduction in carpal range of motion for the ponies receiving level 2 PRFT than for the ponies with arthritis that were not treated.

Table V compares the summarized data between the two groups of ponies receiving amphotericin B on days 1, 4 and 18. Significant differences were present between ponies of the treated group (A_2T_2) and the untreated control group (A_2T_0) in the areas under the lameness-duration curve, range of motion-duration curve, the carpal circumference-duration curve, and the radiographic change-duration curve. The treated ponies showed less lameness (Fig. 5), less swelling of the carpus (Fig. 6), less reduction in range of motion (Fig. 7), and less severe radiographic changes (Fig. 8).

No significant differences were present, for any test parameter, between the treated and untreated legs in the ponies of the A_0T_1 and A_0T_2 groups (Table VI). Based on these results, it appears that PRFT did not have any detectable deleterious effects to the normal joints.

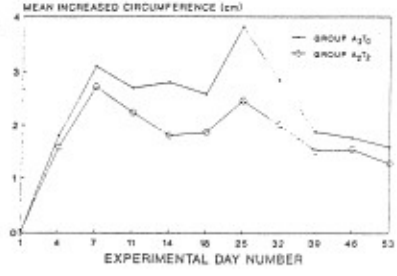

Fig. 6. Test parameter-duration curves showing the mean increase in right carpus circumference that occurred in the ponies receiving amphotericin B on days 1, 4 and 18.

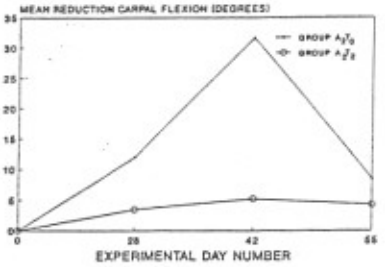

Fig. 7. Test parameter-duration curves showing the mean reduction in right carpus range of motion which occurred in the ponies receiving amphotericin B on days 1, 4 and 18.

DISCUSSION

Significant beneficial effects were apparent when PRFT was used on the MCJ with induced arthritis. The ponies which received 10 mg of amphotericin B gained more benefit from the low dose (level 1) PRFT than from the higher dose treatment. The mucin clot, duration of lameness, area under the lameness-duration curve, radiographic changes, synovial acid phosphatase, and gross pathological changes all indicated that the ponies receiving the level 1 therapy had less inflammation and less pain associated with the MCJs.

Normally, increased synovial protein and decreased range of motion occur as joint inflammation progresses. However, in the treated ponies receiving the single dose of amphotericin B (groups A_1T_1 and A_1T_2) a reversal of this trend occurred. The synovial protein level was significantly lower, and the range of motion was significantly greater, in the treatment group (A_1T_1) having greater joint inflammation, as measured by synovial acid-phosphatase, radiography and gross pathology.

Figures 5, 6 and 7 demonstrate that the differences between the treated and control groups with regard to lameness, joint circumference and range of motion, tended to diminish after the PRFT was stopped at day 30. This could be the result of a diminishing residual benefit of PRFT. Alternatively, PRFT may suppress the normal inflammatory responses, and at some time after the treatment is stopped, the response curves start to parallel those for the untreated animals.

The presence of inflammatory by-products (lysosomal enzymes, prostaglandins, interleukins and free radicals) within the joint are known to cause cartilage and bone changes (1). The reduction in pathological changes noted in the MCJs of the ponies in the A_1T_1 group compared to the control group (A_1T_0) ponies may be the result of treatment causing a reduction of inflammatory by-products, rather than a direct effect of PRFT on the tissues. The development of more severe degenerative changes in the ponies receiving three intra-articular amphotericin B infections is probably the result of a more severe and prolonged inflammatory insult.

Gross pathology scores were based on the assessment of all joint surfaces and tissues, while the histopathological scores were based upon the assessment of a specific area selected for its predilection for changes in naturally occurring DJD. The lack of histological changes seen, relative to the degree of gross pathological lesions noted, may be the result of inadequate sampling from each MCJ.

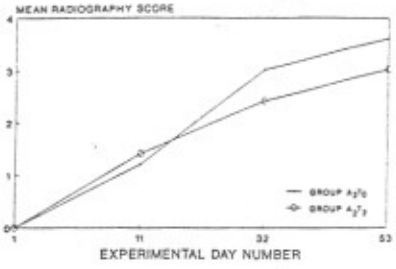

Fig. 8. Test parameter-duration curves showing the mean changes in radiographic lesions occurring in the right middle carpal joint of ponies receiving amphotericin B on days 1, 4 and 18.

The joints which developed degenerative changes in the ponies of the A_2T_0 and A_2T_2 groups were similar, but not identical, to the type of joint disease that would be presented for PRFT in a clinical setting. In clinical practice, it is more likely that the inflamed or degenerative state would have been present for some time prior to the patient being presented for treatment, and a greater degree of injury to the cartilage and subchondral bone would be present. Further

TABLE VI. Comparison of areas under the test parameter curves, and test parameter scores, for groups receiving PRFT on the right carpus. Synovitis was not induced in these groups.

Test parameter	Group A_0T_1 (n = 4) Mean difference ± sd	Significance level	Group A_0T_2 (n = 3) Mean difference ± sd	Significance level
Synovial protein (mg/dL)[a]	-1.94 ± 13.51	ns	-7.46 ± 14.18	ns
Synovial white cells (cells/μL)[a]	-5287 ± 11433	ns	-2483 ± 7944	ns
Mucin clot (score)[a]	0.00 ± 0.00	ns	-2.67 ± 5.16	ns
Carpal circumference (cm)[a]	15.17 ± 41.09	ns	4.35 ± 22.66	ns
Lameness (score)[a]	3.38 ± 8.71	ns	0.00 ± 0.00	ns
Radiology changes (score)[a]	2.62 ± 6.78	ns	5.00 ± 9.68	ns
Reduced range of motion (degrees)[a]	-186.06 ± 523.78	ns	-119.73 ± 194.07	ns
Acid phosphatase (Sigma units)[a]	-25.01 ± 70.24	ns	-40.23 ± 103.38	ns
Gross pathology changes (score)[b]	0.00 ± 0.00	ns	-0.33 ± 1.04	ns
Histopathology changes (score)[b]	0.25 ± 0.65	ns	0.00 ± 0.00	ns

[a]The mean ± standard deviation shown are for differences in areas, i.e. right leg minus the left leg, under the test parameter-experimental days curve. Units of area are: Units of test parameter x days

[b]The mean ± standard deviation shown are for differences in the mean score between the right and left legs. There is no area under the parameter curve

Group A_0T_1: Received: 1 PRFT on the right carpus

Group A_0T_2: Received 2 PRFT on right carpus

investigations are indicated to determine if a beneficial effect can be obtained when chronically affected joints are treated with PRFT.

Inducing arthritis in horses by creating chemical injury to the joint tissues has been described using intra-articular injections of the polyene antibiotics, filipin and amphotericin B (13, 17, 18), Freund's adjuvant (19), and sodium monoiodoacetate (11). None of these experimental models completely reproduce the clinical and pathological changes seen in naturally occurring equine DJD. Amphotericin B was selected for this study because we wished to create an arthritis model that would exhibit lameness for several weeks following a single intra-articular injection. Reports of studies using filipin (17, 18) indicated that a reasonably consistent degree of lameness could be achieved and maintained by intra-articular injections of the drug at weekly intervals. In order to provide a longer period, and greater degree of lameness, for a more severe arthritis model, amphotericin B was used in the three injection series described.

In the A_2T_0 and A_2T_2 group ponies, it was necessary to conduct the lameness examinations on a twice-weekly basis for the first 4 wk because the degree of lameness increased following each amphotericin B injection. One week after the third intercarpal amphotericin B injection, when the degree of lameness started to decline, lameness evaluations were done on a once-weekly schedule.

The purpose of the data manipulation was to determine the changes that occurred in the test parameters as a result of the PRFT. The inflammatory response to arthrocentesis alone may have caused some deviation from normal values to occur. The effects of arthrocentesis alone are removed by subtracting the changes occurring in the left carpus from the changes occurring in the right carpus, providing a more accurate measure of the parameter changes which are the result of the PRFT.

In the assessment of therapy for joint disease, there are two important considerations: the degree and duration of abnormality. The area contained under the test parameter-duration curves combines both of these considerations and provides a simple method to compare treatment methods over a period of time.

Six strides were selected as the minimum number to analyze for determining the range of motion, based on information which states that at least five strides are required to accurately describe gait characteristics in the trotting horse (20). The postinjection range of motion (R_n), was consistently less than the initial range of motion in (R_0) in 24 of the 28 ponies receiving amphotericin B in the right MCJ. This resulted in a negative value for the net data value, Y_n, calculated from the equation, $Y_n = R_n - R_0$. We suspect that adaptation, or learning to trot on the hot walker, resulted in L_n being greater than L_0 in many of the nonarthritic joints. If the range of motion in the left carpus (L_n-L_0) had been subtracted from the range of motion in the right carpus, a larger negative number would result, indicating a greater decrease in the change of right carpal range of motion. Since the effect of learning on the range of motion of the right carpus could not be quantified, and therefore could not be subtracted it would be misleading to subtract the left carpus range of motion from the right in the ponies which had amphotericin B injections into the right MCJ.

All the recorded lamenesses were in the right forelimb. Therefore, all "L" values in the formula:

$$Y_n = (R_n - R_0) - (L_n - L_0)$$

had a value of zero. In the absence of a predominant right leg lameness, an "L" score may have been present. Intra-articular anesthesia would be necessary to remove the predominant right forelimb lameness and determine if a less severe left forelimb lameness was present. However, intra-articular anesthesia was not possible because of the effect the anesthetic drug might have on subsequent synovial fluid samples.

At this time, there is no scientific knowledge available to explain the mechanisms by which PRFT provides the beneficial effects noted in this study. However, the mechanism for delivery of energy to tissues is understood. In the equipment used in this study, a generator develops a radio frequency, high voltage, alternating current wave form with an amplitude of 4500 volts from peak to peak and a frequency of 434 KHz. The high voltage wave form is pulsed on and off every 1/1000 s, i.e. it is nearly 100% amplitude modulated by a 1 KHz pulse train. The power output is kept relatively low by maintaining a substantial phase difference between the maximum voltage and maximum current. When this power is delivered to the output coil of the equipment, high voltage causes ionization of the air, producing a visible discharge, or corona. The corona induces an electrical current of 22 milliamps within the tissues, resulting in the dissipation of approximately 23 milliwatts of energy.

Pulsing a high frequency voltage, 434 KHz in this case, at a low frequency (1 KHz), produces an electrical field having characteristics of both high and low frequency fields. A trait of high frequency fields is that they concentrate and dissipate their energy, in areas of low impedance. *In vitro* tissue temperature studies have been conducted with PRFT to satisfy United States Federal Drug Administration safety requirements for investigational devices. However, histological studies are indicated to determine if there are significant physical or functional changes to low impedance tissues as a result of exposure to PRFT, and if there is a dose related effect upon these tissues.

Radio frequency electrical fields have been used for noninvasive destruction of nerve tissue (21-23) and tumor necrosis (24). Transcutaneous electrical nerve stimulation (TENS) is an established clinical technique to relieve chronic pain (25-29). Although studies have been conducted which relate the beneficial effects of TENS therapy to blood flow (30) and suppression of C-fiber function (31), its mechanism of action remains unknown. Neither of these modalities acts in the same manner as PRFT except that they all use electrical energy delivered to the tissues to create their effect. Tissues from PRFT subjects should have further histological and biochemical examination and membrane function studies conducted to determine which specific types of tissue are affected by the application of pulsed radio frequency energy.

Therapeutic ultrasound differs from PRFT. The benefits derived from use of the former in physiotherapy are due to a vibrating piezoelectric crystal generating an acoustic wave which causes micro-movement of the tissues. Analgesia is considered to be a benefit of hemodynamic changes. However, in a study to quantify the analgesic capabilities of therapeutic ultrasound, it was shown that increased temperature at the skin-transducer interface increased the threshold of skin pain perception as a result of a thermal interaction with the sensory system (32). The effect of pulsed radio frequency energy on the temperature of sensory receptor areas should be investigated.

A nonchemical, noninvasive treatment would be a valuable addition to the veterinarian's armamentarium for the management of DJD. The results of this study, using an experimental model of synovitis/degenerative joint disease, indicate that there are significant beneficial effects to be obtained from PRFT. However, further studies are indicated to provide dose-response information. Experimental studies should be conducted on a chronic DJD model, and controlled clinical trails are necessary to determine which naturally occurring arthritic conditions may benefit from PRFT.

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