PROSPECTIVE EVALUATION OF INTRAARTICULAR DEXTROSE
PROLOOTHERAPY FOR TREATMENT OF OSTEOARTHRITIS IN DOGS

by

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Abstract

The objective of this study was to evaluate the effects of intraarticular dextrose prolotherapy on osteoarthritis of the elbow or stifle in dogs. This was a randomized, double-blind, placebo controlled prospective trial of intraarticular dextrose prolotherapy given at 0 and 6 weeks for relief of osteoarthritis. Dogs with unilateral lameness were evaluated by orthopedic exam, visual lameness score, Canine Brief Pain Inventory (CBPI), goniometry, and by kinetic gait analysis at 0, 6 and 12 weeks. Joint radiographs were scored at 0 and 12 weeks. Ten client-owned dogs with naturally occurring osteoarthritis of the elbow or stifle were enrolled. Initial visual lameness, age, body weight, duration of lameness, and CBPI scores did not differ between groups. Change in CBPI PS score in the prolotherapy group from week 6-12 was significantly less improved than placebo with no other significant differences in CBPI Pain Severity (PS) or Pain Interference (PI) scores between groups. There were no significant differences for range of motion or radiographic scores between groups at any time. Kinetic forces improved in prolotherapy dogs, but were not significantly different between treatment groups at any time. There were no significant benefits of intraarticular dextrose prolotherapy for treatment of osteoarthritis of the elbow and stifle in dogs in this study. Larger enrollments and more stringent inclusion criteria should be considered in future evaluations of prolotherapy.
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List of Abbreviations

OA – Osteoarthritis
IGF-1 – Insulin-like Growth Factor 1
IGF-2 – Insulin Like Growth Factor 2
IGF – Insulin-like Growth Factor
TGF-β – Transforming Growth Factor Beta
IL-1 – Interleukin 1
IL-17 – Interleukin 17
IL-18 – Interleukin 18
TNF- α – Tumor Necrosis Factor Alpha
MMPs – Matrix Metalloproteinases
PROLO – Prolotherapy
PRP – Platelet Rich Plasma
bFGF – Basic Fibroblast Growth Factor
mM – Millimolar
MCL- Medial Collateral Ligament
ACL – Anterior Cruciate Ligament
WOMAC – Western Ontario and McMaster Universities Osteoarthritis Score
KPS – Knee Pain Scale
QOL – Quality of Life
MRI – Magnetic Resonance Imaging
VAS – Visual Analogue Scale
CBPI – Canine Brief Pain Inventory
PS – Pain Severity
PI – Pain Interference
PSW – Pressure Sensing Walkway
PVF – Peak Vertical Force
ROM – Range of Motion
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Dedication

I would like to dedicate this thesis to my loving wife, Giovanna, son, Braden and daughter, Briley for their unwavering support and understanding during my residency. Without their sacrifice and encouragement, such an endeavor would not have been possible.
Chapter 1 - Dextrose Prolotherapy Literature Review

Pathogenesis of Osteoarthritis - A Brief Review

Osteoarthritis is a common and debilitating disease that often affects dogs, humans and other species. The term arthritis encompasses both the conditions of degenerative arthritis as well as osteoarthritis (OA). The term OA is used synonymously with degenerative joint disease, degenerative osteoarthritis, and osteoarthrosis and represents a progressive articular deterioration and attempt to repair diseased tissue. In dogs, the development of OA is most commonly secondary to a primary underlying disease with rupture of the cranial cruciate ligament, hip dysplasia, elbow incongruity, joint laxity or articular fractures usually implicated in initiation of this process. (1) Osteoarthritis is the most common form of arthritis and is thought to affect approximately 20% of dogs and up to 60% of cats. (2,3) OA is also a significant disease in humans and has been found to be a leading cause of disability in people over 60 years of age. (4) Patient specific factors such as age, sex, neuter status, gender, genetics, body weight and condition, and exercise may determine both the susceptibility to, and morbidity associated with, the development of OA in dogs and humans. It seems that predictive values for these factors vary widely among studies. (5-10)

The pathogenesis of osteoarthritis is multi-factorial and complex in both humans and dogs. While the hallmark articular features of OA are cartilage degradation and synovitis, other components of this disease include alterations in subchondral bone and cartilage metabolism, bony proliferation in and around the joint space, fibrosis, and cell signaling by various cytokines
and inflammatory mediators. (11) OA may contribute to musculotendinous pathology leading to loss of function, propagation of OA and increased morbidity.

Progression of osteoarthritis involves three overlapping stages. Early on, cartilaginous extracellular matrix degradation occurs with an increase in water content, decrease in the size of aggrecan molecules, and damage to the collagenous network. (12) Damage to collagen leads to fragility and fibrillation of cartilage. Stage two involves chondrocyte compensation through increased cellular proliferation and metabolic activity. Lastly, stage three of osteoarthritis involves an inability of the chondrocytes to maintain adequate repair efforts leading ultimately to full-thickness cartilage loss. (1)
Osteoarthritis – The Role of Inflammatory Mediators, Cytokines and Growth Factors

Canine chondrocytes and synovial cells produce numerous cytokines and growth factors, which lead to either catabolism or anabolism of cartilage. (13,14) In dogs with naturally-occurring or experimentally-induced cranial cruciate ligament deficiency an increase in cartilage thickness and extracellular matrix proliferation occurs during the first 1-3 years after insult due to the compensatory response. (15,16) Synovial cells, osteoblasts, chondrocytes and macrophages are known to contribute to production of both anabolic cytokines such as Insulin-like Growth Factor 1 and 2 (IGF-1, IGF-2) and Transforming Growth Factor-β (TGF-β), as well as catabolic cytokines such as Interleukins 1, 17, 18 (IL-1, IL-17, IL-18) and Tumor Necrosis Factor-alpha (TNF-α). (17,18) Catabolic cytokines are implicated in cartilage degradation through stimulation of matrix metalloproteinases (MMPs). (17,18) Insulin-like Growth Factors (IGF) and TGF-β have been shown to stimulate aggrecan and collagen synthesis with studies showing decreased IGF and TGF-β in cranial cruciate ligament disease in dogs. (18,19) Prostaglandins, reactive oxygen and nitrogen intermediates such as nitric oxide have also been implicated as messengers, possibly in both the catabolic and anabolic pathways of osteoarthritis with modulation potentially useful for treatment of osteoarthritis. (20, 21) The synovium is also responsible for production of inflammatory mediators and cytokines. An increase in synovial cells, lymphocytes and macrophages has been documented in both induced and naturally occurring OA in dogs. (22,23) Synovial macrophages are thought to play a role in cartilage metabolism by their production of degradative cytokines such as IL-1 and TNF-α and experimental reduction in synovial macrophages decreases production of matrix metalloproteinases and subsequent cartilage aggrecanolysis. (23,24)
Definition of Prolotherapy

Prolotherapy (PROLO) is considered a regenerative therapy and is used to treat musculoskeletal, as well as other types of pain. It was initially described by Dr. George Hackett in the 1950s and may have been in clinical use much earlier. (25) Prolotherapy, also previously termed sclerotherapy or regenerative injection therapy, involves injection of a “proliferant” solution into diseased or injured tissue to incite an acute and enhanced inflammatory response with increased healing of damaged tissues. (26) The technique involves periodic intraarticular, peri-articular, tendinous, ligamentous, or peri-spinal injections. Proliferants are broadly characterized as irritants, particulates, osmotics, chemotactics or biologics. (26) Solutions used include osmotics such as dextrose, irritants such as phenol, chemotactics including sodium morrhuate and biologics such as platelet-rich plasma (PRP), stem cells, or autologous whole blood. (26, 27) Most commonly, hyperosmolar solutions of 12.5-25% dextrose have been evaluated experimentally and clinically. (28)
Proposed Mechanism of Action for Dextrose Prolotherapy

Numerous theoretical mechanisms of action for prolotherapy have been described. The anabolic response of cartilage in OA is believed to represent an innate attempt to repair diseased cartilage, which theoretically mimics the usual process of wound healing and thus may be enhanced by various proliferative treatments. (26) Proponents of prolotherapy purport that injection of various substances into diseased joints, tendons or ligaments incites an inflammatory response leading to healing through inflammation, granulation tissue formation and maturation with collagen formation. (26) Initiation of the inflammatory cascade involves osmotic, or other types of cell injury, release of intra-cellular enzymes and attraction of granulocytes for the purpose of debridement. Macrophages and monocytes soon arrive and secrete various cytokines and growth factors that are chemotactic for fibroblasts. Macrophages have been shown to carry out cellular debridement and recruitment of fibroblasts with secretion of collagen to eventually form a tighter, thicker ligament via crosslinking and dehydration. Photomicrographs confirm the presence of granulation tissue at prolotherapy injection sites. (29-34) This granulation tissue ultimately matures and produces collagen in the maturation phase. (26,35) In summary, dextrose prolotherapy may lead to an exaggerated inflammatory response, improved collagen production and healing of tissues.
Dextrose Prolotherapy- *In Vitro* and *In Vivo* Studies

It is often stated that dextrose prolotherapy incites production of numerous growth factors and inflammatory cytokines. Several *in vitro* and *in vivo* studies have evaluated treatment effects of different growth factors. Cultured human chondrocytes exposed to TFG-β, IGF-1 and basic fibroblast growth factor (bFGF) have been shown to exhibit cellular proliferation. (36,37) Intraarticular stifle injection of TFG-β and bFGF in lab animals has been associated with cartilage proliferation and repair. (38,39) *In vivo* experimental studies have also evaluated hyperosmolar dextrose injections utilizing histopathology, mechanical testing and other criteria. Injection of 20% glucose has been reported to cause osmotic shock, localized tissue trauma, and an inflammatory reaction that proceeds along the usual phases of wound healing with attraction of granulocytes, production of prostaglandins, chemical debridement at the injection site and recruitment of macrophages. (26,30) Administration of extracellular glucose as low as 0.5% has been shown to raise levels of multiple polypeptide growth factors in a variety of human cells. (40-44) Specifically, dextrose concentrations of 0.5% have been shown to stimulate human fibroblast and chondrocyte production of TGF-β and IGF with promotion of type 1 and 3 collagen in tenocytes. (45-47) Human osteoarthritic synovial tissue exposed in vitro to 5.5 millimolar (mM) (178% solution) glucose did have a two-fold increase in hyaluronic acid production unlike cells exposed to 0.5 mM glucose solution. (48)

Several studies have evaluated dextrose prolotherapy specifically in terms of effects on cartilage and progression of OA. A intraarticular solution containing 10% glucose, dextrose, amino acids and ascorbic acid was compared in blinded fashion to saline control in rabbits with transected anterior cruciate ligaments. (49) With established OA and treatment performed over a 13-week period, rabbits receiving the nutritive mixture showed significantly improved
histopathologic cartilage scoring, better restoration of the extracellular matrix and inhibition of OA progression as compared to control. (49) This study has cofounding factors of inclusion of amino acids and ascorbic acid in the test injections.

Jensen, et al investigated dextrose, sodium morrhuate and phenol-glycerine-glucose injections in medial collateral ligaments (MCL) of rats and documented the presence of variable tissue inflammation in dextrose-treated subjects but failed to show that the inflammatory response differed significantly from dry needling or saline injections. (50) Further work by the same author using stretch-injured MCLs in rats found statistically significant increase in MCL cross-sectional area of 30% and 90% compared with saline and non-injured controls given two weekly injections. This study found no changes in dextrose treated subjects in regards to strength or stiffness of the ligament and concluded that clinical improvement with dextrose prolotherapy may not result from improvement in ligament biomechanics. (51) More recently, a total of 3 injections of 12.5% dextrose given at 5 day intervals into rat Achilles tendons were compared to corticosteroid or saline placebo with no significant differences found with respect to maximal load at failure and absorbed energy. (52) Histopathology found statistical significance for increased presence of lymphocytic inflammation, a lack of significant difference for neovascularization and increased fibroblasts and concluded that while dextrose was not deleterious to tendons it failed to change mechanical or histologic properties. (52)
Clinical Use of Dextrose Prolotherapy for Osteoarthritis in Humans

Numerous blinded and partially blinded clinical trials and both controlled and non-controlled studies evaluating dextrose prolotherapy exist in the human literature. In a randomized, double-blind, placebo-controlled trial, prolotherapy in the form of 10% dextrose (611 mOsm/L), 0.75% lidocaine and bacteriostatic water (105 mOsm/L) injected in osteoarthritic human knees at 0, 2 and 4 months led to clinically and statistically significant improvements in knee osteoarthritis as measured in terms of pain at rest, pain with walking, pain with stair use, swelling, buckling episodes and flexion range at 6 months after starting treatment. Using these criteria, dextrose exhibited a statistically superior effect to a placebo of 0.75% lidocaine in bacteriostatic water. (53) Control group interventions and evaluations were discontinued 6 months after study enrollment and data at this time revealed no statistically significant difference between treatment and controls in regards to Anterior Cruciate Ligament (ACL) laxity. In the subjects receiving dextrose prolotherapy, treatment was continued for a total of 12 months with 3 additional bi-monthly injections with reported improvement in pain of 40%, swelling by 63%, buckling episodes by 85% and flexion by 14 degrees as compared with values at the time of enrollment. (53) Blinded radiographic grading of osteoarthritis and cartilage thickness one year post-enrollment found a statistically significant change from baseline in scoring for the dextrose-treated knees but did not include control group comparison. (53) ACL laxity, when present, improved by 12 months of initiation of therapy. (53) The primary limitation of this study was varying degrees of osteoarthritis in treated subjects, lack of pre-treatment severity of OA designation and advanced imaging to determine presence of complete ACL rupture.

In a continuation of the study above by Reeves, et al, non-blinded, non-controlled and non-randomized evaluation of the long-term effects of dextrose prolotherapy for patients with
ACL laxity was performed. Patients received intraarticular injection of 6-9 cc of 10% dextrose (400mOsm/L) at 0, 2, 4, 6 and 10 months and 6cc of 25% dextrose at 12 months and it was found that patients experienced clinically and statistically significant improvement from baseline in ACL laxity, pain, swelling and knee range of motion at 12 and 36 months with slightly less post-injection discomfort in the 10% dextrose cohort. (54) While this study did utilize a previously validated objective measure of ACL deficiency-induced laxity, it lacked rigor in both design, objective outcome measures and was limited in power.

In an uncontrolled study, Rabago et al evaluated 36 patients with moderate to severe knee OA and reported improvement utilizing the previously validated Western Ontario McMaster University Osteoarthritis Index (WOMAC) patient quality-of-life assessment and Knee Pain Scale (KPS) for function and stiffness scores with one-year follow-up. (55)

In a subsequent randomized, blinded, placebo-controlled trial of patients with radiographically assessed moderate knee OA, the same author compared intra- and extra-articular dextrose injections to saline control and exercise alone at 1, 5 and 9 weeks using WOMAC, KPS, procedural-related pain, and treatment satisfaction. (27) Dextrose prolotherapy resulted in significant improvement in treated subjects as compared to saline injections or exercise alone. WOMAC scores at 52 weeks showed statistically significant improvement in 50% of dextrose-treated patients as compared to only 30% and 24% for saline and exercise groups respectively. Similar significant differences comparing dextrose injections to saline or exercise were also noted at 9 and 24 weeks. WOMAC and KPS scores for function and pain respectively were also significantly better in the dextrose group at both 9, 24 and 52 weeks. (27) Rabago et al also evaluated patients with knee OA for quality of life (QOL) and intraarticular cartilage volume utilizing WOMAC and magnetic resonance imaging (MRI) in partially-blinded
and controlled fashion. (56) Knee related QOL scores in dextrose prolotherapy treated patients surpassed controls (p=0.05) at 52 weeks. Both groups lost cartilage volume over time with no difference between groups and those with the lowest decrease in cartilage volume having the highest QOL scores. It was concluded that in prolotherapy patients, MRI-assessed cartilage stability predicted improvement in WOMAC pain scoring and that prolotherapy may lead to decreased pain via sensorineural effects. (56)

Dumais, et al administered 15 and 20% dextrose in extra and intraarticular fashion and rehabilitative exercise in 45 humans with knee osteoarthritis utilizing an open-label, crossover, non-placebo controlled randomized study. (57) While their study found an overall 29% improvement in WOMAC scores for patients given dextrose, but not for exercise without injection, the study suffered from low power, lack of blinding and absence of placebo. (57)

In a prospective, double-blind evaluation of dextrose prolotherapy for osteoarthritic thumb and finger joints, it was found that 0.5 ml/site of 10% dextrose with 0.75% xylocaine in bacteriostatic water given intraarticularly at 0, 2 and 4 months led to statistically significant improvements in pain with movements of fingers as well as flexion range of motion as compared to a placebo of 0.075% xylocaine in bacteriostatic water. (58) Outcome measures included use of a 100mm Visual Analogue Scale (VAS) for evaluation of pain at rest and with joint movement and goniometry in flexion. (58) Double-blind assessments of VAS at 6 months found an averaged 37% improvement in VAS at rest, on movement, and for grip pain in the dextrose-treated joints compared to 18% in placebo-treated but only decreased pain with movement was statistically significant. (58) Treatment was deemed clinically effective in the treatment of pain, safe, and side effects were reported to be minimal. An average of 45% improvement in pain level was noted in the dextrose group from 6-12 months. At 12 months, the authors instituted double-
blind administration of dextrose to patients previously in the control group with averaged joint pain reduction from 18% in the control group to 54% once treated with dextrose. (58) Patients were unaware of treatment given throughout the study. Primary limitations included subjective outcome measures, failure to reach significance for several outcome variables, and small sample size (n=25).
Veterinary Literature

Veterinary reports of dextrose PROLO are currently limited to isolated case reports, conference proceedings and clinical reviews with no published scientific clinical trials or structured case series. (59-62) Clinical reports with only subjective and individual patient outcomes reported by these authors indicate treatment success using various forms of dextrose prolotherapy for spinal cord injury, cranial cruciate ligament deficiency, coxofemoral osteoarthritis, hip laxity and patellar luxation but fail to utilize any outcome measures. (59-61,63) Specifically, one case series reports successful treatment of each of the following in different dogs: “degenerative myelopathy and hip dysplasia, trauma to the patella, loss of hind end stability, partial repair of the anterior cruciate ligament, post-operative cranial cruciate ligament pain and severe hip dysplasia” although several patients also received laser and ultrasound therapy, and acupuncture (59). This case series does not mention substances used, sites injected or outcome measures (59). One author claims success rates “in the vicinity of 90% using a mixture of 25% of each of the following: 50% dextrose, 2% lidocaine or procaine, vitamin B 12 (1000 mcg/mL) and homeopathic combinations such as Biosode Support, Traumell, or other compounds for treatment of cranial cruciate ligament disease (63). According to this author, this mixture is often used in conjunction with chiropractic care, laser therapy and acupuncture. (63) In summary, case reports and the one veterinary literature review regarding prolotherapy only support the fact that prolotherapy has been used with varying techniques and dosages for the above conditions. At the time of this writing, there are no published, peer-reviewed clinical trials evaluating dextrose prolotherapy in dogs.
Conclusions

Osteoarthritis is a prevalent and debilitating disease both in the human and veterinary populations. Prolotherapy is often used as an adjunctive or last-resort treatment for humans suffering from various forms of OA or musculotendinous disorders. The actual mechanism of action for dextrose prolotherapy remains unproven. Currently, there are numerous clinical studies with varying levels of evidence for selection of prolotherapy as a non-invasive and economical modality for treatment of refractory OA. While several studies supporting efficacy for this treatment suffer from lack of blinding or inclusion of control groups, more recent works with appropriate study design do suggest efficacy of dextrose prolotherapy for knee OA in humans. Results of recent human clinical studies are encouraging but further rigorous evaluation in different disease conditions is warranted prior to acceptance of this alternative therapy.
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Chapter 2 - Prospective Evaluation of Intraarticular Dextrose Prolotherapy for Treatment of Osteoarthritis in Dogs

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“Prospective Evaluation of Intraarticular Dextrose Prolotherapy for Treatment of Osteoarthritis in Dogs”

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Abstract

Objective: To evaluate the effects of intraarticular dextrose prolotherapy on osteoarthritis of the elbow or stifle in dogs.

Methods: This was a randomized, double-blind, placebo controlled prospective trial of intraarticular dextrose prolotherapy given at 0 and 6 weeks for relief of osteoarthritis. Dogs with unilateral lameness were evaluated by orthopedic exam, visual lameness score, Canine Brief Pain Inventory (CBPI), goniometry, and by kinetic gait analysis at 0, 6 and 12 weeks. Joint radiographs were scored at 0 and 12 weeks.

Results: Ten client-owned dogs with naturally occurring osteoarthritis of the elbow or stifle were enrolled. Initial visual lameness, age, body weight, duration of lameness, and CBPI scores did not differ between groups. Change in CBPI PS score in the prolotherapy group from week 6-12 was significantly less improved than placebo with no other significant differences in CBPI Pain Severity (PS) or Pain Interference (PI) scores between groups. There were no significant differences for range of motion or radiographic scores between groups at any time. Kinetic forces improved in prolotherapy dogs, but were not significantly different between treatment groups at any time.

Clinical Significance: There were no significant benefits of intraarticular dextrose prolotherapy for treatment of osteoarthritis of the elbow and stifle in dogs in this study. Larger enrollments and more stringent inclusion criteria should be considered in future evaluations of prolotherapy.
Introduction

Osteoarthritis (OA) affects 20% of the canine population and remains a debilitating and costly disease by quality of life impairment and owner expense. In dogs, OA is often secondary to injury or congenital abnormality and consists of overlapping stages of extracellular matrix degradation, chondrocyte proliferation and chondrocyte and cartilage loss. Regenerative medical therapies are commonly used in humans to palliate OA pain and have also been reported in dogs. Regenerative medicine therapy, as defined by the U.S. National Institute of Health, is the “process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects.” (1) Examples include stem cells, platelet-rich plasma, prolotherapy and other modalities that seek to influence inflammation, tissue proliferation and modulation of OA and other disease processes.

Prolotherapy (PROLO) is a regenerative therapy with “proliferants,” classified as irritants, particulates, osmotics, chemotactics or biologics, injected into diseased joints, tendons, ligaments or a para-spinal area with the intent to provoke an inflammatory response and increased proliferation of tissues during repair. (2,3) Injections of concentrated dextrose, phenol, platelet-rich plasma (PRP), stem cells, autologous whole blood, sodium morrhuate, and dry needling, have been used for PROLO. (4) Dextrose PROLO, the most commonly reported agent in human medicine, is hypothesized to cause localized tissue trauma due to osmotic shock which results in inflammation, subsequent production of numerous prostaglandins and growth factors, cellular proliferation and finally, a reduction in inflammatory interleukins. (5-10) In an in vitro model utilizing pre-osteoblasts and patellar ligament fibroblasts exposed to varying concentrations of a phenol-dextrose solution, increased collagen production was observed. (5) In vivo experimental models have shown no changes in ligament maximum load to failure or energy
absorption when dextrose was injected into rat Achilles tendons. (11) Other studies have found increased cross-sectional area with unchanged laxity in stretch-injured rat medial collateral ligaments, and restorative effects on the cartilage matrix in an anterior cruciate ligament transection model in rabbits. (12,13)

In human medicine, administration of dextrose PROLO has been reported for treatment of osteoarthritis, knee instability, meniscal pathology, tendon injury, and various forms of spinal pain. (14-18) Systematic reviews in the human literature have found varying levels of efficacy for treatment of musculoskeletal conditions with dextrose PROLO and none reported significant adverse effects. (4,19,20) While numerous clinical trials utilizing PROLO for OA in humans exist, controlled, blinded, randomized trials are still currently lacking. (19) Two recent controlled studies of human knee OA did find that dextrose PROLO resulted in safe, substantial improvement in specific knee-osteoarthritis quality of life outcome measures such as pain, stiffness, function and symptom severity in treated patients as compared to saline control injections when evaluated by the Western Ontario McMaster University Osteoarthritis Index. (2,21) Veterinary reports of dextrose PROLO are limited to isolated case reports, conference proceedings and clinical reviews with no published methodological scientific research. (20,22-24)

The purpose of this study was to evaluate the effects of intraarticular 25% dextrose prolotherapy for treatment of naturally-occurring osteoarthritis of the elbow or stifle in dogs. Evaluation was by veterinary lameness exam, (25) a previously validated owner pain survey, (26) goniometry, and a pressure sensing walkway (PSW) utilized as outcome measures. Our hypothesis was that dogs receiving PROLO injections would show improved veterinary lameness scores, better range-of-motion and improved weight bearing of the affected limb.
**Materials and Methods**

This was a randomized, double-blind, placebo controlled clinical prospective trial designed to test the clinical effectiveness of dextrose PROLO in the relief of lameness and pain in dogs with naturally occurring osteoarthritis. This study was approved by the Kansas State University Institutional Animal Care and Use Committee. The study population consisted of client-owned dogs presenting to the Kansas State University Veterinary Health Center (KSU-VHC) for evaluation and treatment of lameness. Costs associated with lameness evaluation and treatment were paid by the AKC Companion Animal Fund and owners received no other financial incentive to participate. Solicitation of patients consisted of electronic communication to referring veterinarians as well as faculty, staff and students of the Kansas State University College of Veterinary Medicine and Veterinary Health Center.

For the purposes of the study, dogs were evaluated at week 0, 6 and 12. Dogs were randomized to treatment or placebo group assignment prior to study enrollment. In order to be eligible for the study, dogs were required to have a body weight > 20kg, have a history of unilateral lameness as reported by the owner and have a minimum of 5% decrease in peak vertical force (PVF) of the lame limb measured as a percentage of body weight (kg) on a PSW.\(^a\) Prior to enrollment, dogs were permitted to be on non-steroidal anti-inflammatories (NSAIDs), dietary supplements for arthritis, therapeutic diets or other analgesics except for corticosteroids, with changes in medications or supplements not permitted for two weeks prior to enrollment or during the twelve-week study period. Exclusion criteria included any dog with a temperament not suited for PSW lameness or orthopedic examination, changes to analgesic medications within 2 weeks of study enrollment or during the study, orthopedic surgery of any limb within 6 months of initial evaluation or failure to exhibit measureable lameness \(\geq 5\%\) as compared to the
contralateral limb on the PSW. Dogs presenting with lameness due to cranial cruciate rupture were only included if owners declined the recommended surgical therapy.

Initial evaluation included a brief owner questionnaire to define the limb affected and duration of lameness, history of any orthopedic surgery, and the type and duration of current pain medications or supplements. Owners were also given the Canine Brief Pain Inventory (CBPI) with the same individual required to complete the survey during each evaluation. The CBPI is a previously validated two-part owner questionnaire evaluating both the pain severity (PS, questions 1-4) and pain interference (PI, questions 5-10) associated with daily activities. (26,27) A complete orthopedic examination and visual veterinary lameness exam were performed with a lameness grade of 0-5 assigned by a single observer (JMS) as previously reported and described in Appendix 1. (25) The dog was walked across a Tekscan PSW by one of two handlers to obtain five valid trials for evaluation of stance time, stride velocity, PVF, vertical impulse, and maximum peak pressure, using system-specific software. Walking velocity was controlled to achieve 1.0-1.9 m/s with a standard deviation of 0.1 m/s. (27,28) If a lameness of ≥5% PVF difference from the contralateral limb was detected, the dog was then sedated with hydromorphone 0.15 mg/kg and acepromazine 0.02 mg/kg IV to obtain orthogonal computed radiographs of the elbow or stifle of the affected joint as determined by orthopedic examination and palpation by a single observer (JMS). If suspicion of other joint involvement on the same limb existed, radiographs of the additional joints were obtained to rule out other causes of lameness. Enrollment was continued if radiographs confirmed osteoarthritis of the affected joint with no evidence of more than one source of arthritis or sources of pain on examination on that limb. Joint radiographs taken at time 0 and 12 weeks were scored for osteoarthritis by a blinded, board certified radiologist (LJA) at the time of study conclusion in a
manner similar to previously reported criteria. (29) Radiographs were scored as follows: normal=0, mild OA=1, moderate OA=2, severe OA=3. Radiographic OA scoring was used to confirm presence of OA and to evaluate any changes in severity at final evaluation.

Goniometry was performed by a single observer (JMS) using a two-arm plastic goniometer\textsuperscript{e} with 1° increments as previously reported. (30) The means of three values for flexion and extension of the affected and contralateral joint were recorded. (30)

**Pre and post-treatment care**

While the dog was still sedated, the affected joint was clipped and prepped with chlorhexidine scrub and an alcohol wash. Aseptic injection of either 5 ml of the PROLO agent, 25% dextrose (4mL sterile water,\textsuperscript{f} 1 mL 2% lidocaine,\textsuperscript{g} 5 mL 50% dextrose\textsuperscript{h}) or the placebo (4mL sterile water,\textsuperscript{f} 1 mL 2% lidocaine,\textsuperscript{g}) was performed by a single blinded investigator (JMS). Intraarticular injection was confirmed by detection of grossly observed joint fluid and joint distension. Dogs were monitored for any signs of post-injection pain for a minimum of 4 hours after treatment and then discharged with instructions to monitor for increased pain, lameness or swelling. No dogs required post-injection pain medications based on clinician or owner assessment.

Dogs were reevaluated at 6 and 12 weeks. Evaluation at week 6 involved the same historical questions, CBPI completion by the same owner, repeat orthopedic examination, visual lameness scoring, PSW evaluation and repeat injection of treatment or placebo while utilizing the same sedation protocol as week 0. The final evaluation at week 12 consisted of all components of week 6 except the intraarticular injection and with the addition of repeat radiographs of the affected joint.
**Statistical Analysis**

Age, body weight at each time period, range of motion (ROM) of the affected joint at each time period, and the duration of lameness were compared between treatment groups by Independent group T-Test. Radiographic osteoarthritis scores were compared at week 0 and at week 12 between treatment groups by nonparametric Mann-Whitney U. The CBPI PS and PI scores were recorded as a numerical total of values assigned to questions 1-4 (maximum 40) and 6-10 (maximum 60), respectively. The CBPI PS, PI and median visual lameness scores were compared at each time period between treatment groups by nonparametric Mann-Whitney U. Change in CBPI PS and PI scores between weeks 0-6, 6-12, and 0-12 were compared by nonparametric Mann-Whitney U. The percent change between weeks 0-6, 0-12, and 6-12 for the parameters of stance time, stride velocity, PVF, vertical impulse, and maximum peak pressure were compared for the treated and contralateral limbs between treatment groups by an independent T-test. A commercial statistical software program was used for all comparisons and $p \leq 0.05$ was considered significant.
Results

Seventeen dogs were evaluated. Ten dogs met inclusion and exclusion criteria and were enrolled. The most common cause for exclusion was failure to exhibit a measureable lameness of ≥ 5% as compared to the contralateral limb (n=6) with one dog eliminated based on suspected neurologic disease. Initial and week 6 data from one placebo dog was included, but week 12 data was lost as the dog died of unknown cause eighteen days after the six week treatment. The injection site was not reported to be abnormal at the time of death so it is believed to be unrelated to treatment. Of the ten dogs, five were randomly allocated to receive PROLO while the remaining five received the placebo. The mean age of all dogs was 5.7 years (median 4.5 years). There was no significant difference in mean age of the PROLO (5.7 years) and placebo groups (7 years, p=0.601). The mean body weight at week 0 for all dogs was 38.4 kg (median 35.8 kg). There was no significant difference for mean body weight of the PROLO (36.58 kg) and placebo groups (40.1 kg, p=0.599). The mean duration of lameness was 19.5 and 9.4 months for PROLO and placebo groups respectively (p=0.350) with no significant difference in initial median veterinary visual lameness scoring between treatment groups (p=1.0). There were no significant differences between PROLO and placebo groups for the initial CBPI PS (p=0.834) or PI scores (p=1.0). Three stifles and two elbows were randomly assigned to the PROLO group while three elbows and two stifles were assigned to the placebo group. Four of five dogs in the PROLO group and one of five placebo dogs had physical exam findings consistent with OA of the contralateral joint with the difference between groups not statistically significant (p=0.058). One PROLO and two placebo group dogs were concomitantly receiving concurrent NSAIDs and nutraceuticals. Age, body weight at each time period, duration of lameness, and ROM at each time period were not significantly different between PROLO and
placebo groups. Overall, median lameness scores at time 0 were 3/5 and 2/5 for PROLO and placebo respectively. From time 0 to time 12, median PROLO lameness scores improved by 1 point for PROLO and did not improve for the placebo group with no significant difference between groups (p=0.391). Median CBPI PS and PI scores are presented in Table 2.1. The change of CBPI PS score in the PROLO group from week 6-12 (median=0) was significantly less improved (p=0.027) than the change of CBPI PS score from week 6-12 (median= -6.5) in the placebo group. There were no other significant differences in the CBPI PS and PI scores between treatment groups at any time period.

There was no significant difference in mean change in ROM at Week 0-6 (p=0.708), Week 6-12 (p=0.424) or Week 0-12 (p=0.393) between PROLO and placebo treated joints (Table 2.2).

Median OA scores for both PROLO and placebo groups were 2 at Times 0 and 12. There were no significant differences in OA scores between PROLO or placebo groups at Time 0 (p=0.754) or at Time 12 (p=0.806).

Kinetic gait data for PROLO and placebo injected limbs are presented in Table 2.3. The stride velocity of the contralateral limb in the PROLO group (-12.15%) decreased significantly compared to that of the contralateral limb in the placebo group (19.94%) from Week 6-12 (p=0.005). For measured stance time, stride velocity, PVF, vertical impulse and maximum peak pressure, there were no significant percentage changes over time in either the treated or contralateral limbs between treatment groups, despite the appearance that PROLO dogs improved more than placebo (Figures 2.1 and 2.2). Post Hoc power analysis indicated that a
sample size of 29 to 106 animals would have been needed to see a significant difference in peak vertical force between treatment groups at various time intervals.
Table 2.1: Median scores for CBI Pain Severity (PS) and CBI Pain Interference (PI) for each group at each time period.

<table>
<thead>
<tr>
<th>Time period</th>
<th>PROLO PS</th>
<th>Placebo PS</th>
<th>PROLO PI</th>
<th>Placebo PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>18</td>
<td>18</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Week 6</td>
<td>12</td>
<td>18.5</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Week 12</td>
<td>16*</td>
<td>13.5*</td>
<td>20</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* Indicate significant differences between treatment groups from week 6-12. There were no other significant differences between treatment groups at p≤0.05.

Table 2.2: Change in Range of Motion (ROM) Over Time and Between Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>CHG ROM 0-6</th>
<th>CHG ROM 6-12</th>
<th>CHG ROM 0-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROLO</td>
<td>-2.6</td>
<td>6.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.4</td>
<td>-2.0</td>
<td>-4.5</td>
</tr>
</tbody>
</table>

P values represent differences between treatment groups at each time period.
<table>
<thead>
<tr>
<th></th>
<th>0-6 (% and S.D.)</th>
<th>6-12 (% and S.D.)</th>
<th>0-12 (% and S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stance Time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROLO</td>
<td>-6.1 ± 11.7</td>
<td>14.4 ± 13.6</td>
<td>7.8 ± 20.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>6.0 ± 18.1</td>
<td>17.7 ± 36.7</td>
<td>14.5 ± 29.1</td>
</tr>
<tr>
<td>p value</td>
<td>0.244</td>
<td>0.875</td>
<td>0.698</td>
</tr>
<tr>
<td><strong>Stride Velocity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROLO</td>
<td>1.6 ± 14.3</td>
<td>4.4 ± 27.2</td>
<td>8.1 ± 42.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>7.7 ± 21.7</td>
<td>-6.3 ± 25.4</td>
<td>-8.8 ± 22.3</td>
</tr>
<tr>
<td>p value</td>
<td>0.614</td>
<td>0.562</td>
<td>0.498</td>
</tr>
<tr>
<td><strong>Peak Vertical Force</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROLO</td>
<td>8.1 ± 25.9</td>
<td>10.5 ± 22.9</td>
<td>24.0 ± 57.0</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.1 ± 5.8</td>
<td>-5.4 ± 16.5</td>
<td>-5.3 ± 16.5</td>
</tr>
<tr>
<td>p value</td>
<td>0.628</td>
<td>0.283</td>
<td>0.358</td>
</tr>
<tr>
<td><strong>Vertical Impulse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROLO</td>
<td>9.9 ± 31.7</td>
<td>28.7 ± 37.5</td>
<td>46.4 ± 75.9</td>
</tr>
<tr>
<td>Placebo</td>
<td>-9.4 ± 36.7</td>
<td>14.7 ± 24.2</td>
<td>-7.6 ± 42.5</td>
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<tr>
<td>p value</td>
<td>0.402</td>
<td>0.542</td>
<td>0.248</td>
</tr>
<tr>
<td><strong>Max Peak Pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROLO</td>
<td>3.1 ± 17.5</td>
<td>1.8 ± 9.1</td>
<td>6.0 ± 26.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>-3.9 ± 5.4</td>
<td>-4.7 ± 6.0</td>
<td>-7.1 ± 10.5</td>
</tr>
<tr>
<td>p value</td>
<td>0.419</td>
<td>0.259</td>
<td>0.391</td>
</tr>
</tbody>
</table>

Results expressed as mean % change ±standard deviation. P values represent comparison between PROLO and placebo groups at each time period.
Figure 2.1: Percent Mean Change in Peak Vertical Force - Percentage mean change and standard deviation in Peak Vertical Force (% body weight) from 0-6 Weeks, 6-12 Weeks and 0-12 Weeks.
Figure 2.2 Percent Mean Change in Peak Vertical Impulse - Percentage mean change and standard deviation in Vertical Impulse (% body weight) from 0-6 Weeks, 6-12 Weeks and 0-12 Weeks.
Discussion

Overall, the results of this study did not find a statistically significant difference in most subjective and objective parameters of lameness evaluated after injection of osteoarthritic joints with dextrose prolotherapy as compared to placebo. The two findings that were significantly different between treatment groups (CBPI PS score and Stride Velocity at Week 6-12) do not support benefit of dextrose prolotherapy as compared to placebo and such sporadic findings are likely a result of normal variable distribution. Dogs in both treatment groups did not exhibit significant differences for criteria of age, body weight, affected joint, use of analgesics and pre-treatment subjective veterinary lameness scoring. Mean duration of lameness was 19.5 and 9.4 months for PROLO and placebo groups respectively. While this difference was not found to be significantly different, the longer duration of lameness could potentially have contributed to a decreased response to PROLO as measured by CBPI results, range of motion, and pressure sensing walkway data. While we could not demonstrate that increased duration of lameness was related to increased severity of OA and morbidity in this study, it is possible that the PROLO dogs may have been more likely to have chronic pain. Likewise, the number of dogs with contralateral limb abnormalities was not significantly greater in the PROLO group (four) as compared to placebo (one), but may have affected veterinary lameness scoring and CBPI results.

There was no significant difference in subjective veterinary lameness scoring between groups at any recorded interval. This would suggest that the longer duration of lameness noted in the PROLO group was inconsequential to outcome. Past studies have found that subjective lameness evaluation is inferior to force plate analysis, unless severe lameness exists. (31) Dogs in the PROLO group showed a greater improvement in lameness score from week 0-12 as compared to treatment but this result was not significantly different. CBPI PS and
PI Scores found that the placebo group showed significant improvement as compared to treatment when evaluated from time 6-12, but there was no significant difference for all other times between groups. Possible explanations for this finding include placebo effect influencing owner survey results, lack of significant treatment effect in the dextrose prolotherapy group, or the small number of dogs evaluated. Recent evaluation of the ability of the CBPI to detect significant improvement in osteoarthritic dogs treated with carprofen found that a minimum pre-treatment inclusion criteria of ≥ 2 for both mean PS and PI scores was necessary to detect improvement with treatment. (27) This same study found that criteria of a decrease in PS of ≥1 and PI of ≥ 2 resulted in the most statistical power to predict if a treatment would lead to response in an individual dog. In the present study, both groups had equivalent total PS and PI scores well above this minimum criteria with only 1 dog in each group having values <2 for either mean PS or PI, or both. Based on published CBPI criteria and our data, our dogs had sufficient lameness to allow for detection of significant treatment effect, had it been present. Interestingly, both PROLO and placebo had mean CBPI PS/PI improvements but the changes were not significant.

PROLO dogs gained an average of 3.4 degrees and placebo dogs lost 4.5 degrees ROM from enrollment to study conclusion. Despite this apparent difference, the overall mean change in range of motion was not significantly different between treatment groups.

We did not find significant differences between ground reaction force change over time but did note significant difference for one kinetic variable (stride velocity) between treatment groups. Though statistically insignificant within the small number of dogs of this study, there were several interesting trends noted. Mean PVF in PROLO limbs increased by 8.1% (S.D. ± 25.9%, p=0.628) and 24%(S.D. ± 57%, p=0.358) at Weeks 6 and 12 respectively.
Although the difference was not significant and standard deviations were large, PVF in placebo-treated limbs only improved 2.1% at Week 6 and then decreased by 5.3% from baseline at Week 12 as compared to Week 0. Similar trends were noted for vertical impulse and maximum peak pressure with lack of significance and wide standard deviations recorded. The fact that the stride velocity of the contralateral limb in the treated group decreased significantly compared to that of the contralateral limb in the untreated group from Week 6-12 is most likely due to transient increased contralateral lameness in the PROLO dogs with known contralateral disease (n=4). This finding may also be consistent with the lack of significant positive treatment effects observed in PROLO treated limbs.

The primary limitation of this study was the small number of dogs and thus, low power achieved. Post Hoc power analysis indicated that a sample size of 29 to 106 animals would have been needed to see a significant difference in peak vertical force between treatment groups at these time intervals. Other confounding variables are those inherent to prospective studies involving dogs with multiple joints affected by OA. While it was our intent to identify and enroll dogs with reported single-limb lameness, many of our patients in fact had contralateral OA and OA affecting both rear and forelimbs. The fact that 4/5 dogs in the PROLO group had contralateral disease may have contributed to the lack of improvement noted. Another potential limitation would be the inclusion of 5/10 (50%) dogs with lameness due to cranial cruciate ligament rupture in the study. The failure to show improvement in this subset of dogs may have been due to continued instability due to ligament rupture. It is possible that subsequent studies limiting enrollment to dogs with OA and no cruciate deficiency, or other cause of mechanical joint instability might find improved results of prolotherapy. However, the population was representative of dogs presenting for management of OA in a practice setting and thus
appropriate for this type of study. One other limitation includes NSAID use during the study. While NSAID use was only present in a small portion of dogs in our study, it’s possible that use could have biased results. Ideally, dogs would have all been taken off analgesic medications and undergone a washout period prior to study enrollment. Although controversial, many practitioners of prolotherapy in humans recommend discontinuing NSAIDs after treatment to prevent inhibition of the desired inflammatory response. (3) We elected to allow dogs to be continued on any previous medical management to avoid increasing their pain. Future studies should include more stringent exclusion guidelines.

Though some dogs did improve after PROLO, this study failed to demonstrate significant benefits of intraarticular dextrose prolotherapy as compared to placebo for treatment of osteoarthritis of the elbow and stifle in dogs. The treatment was well-tolerated and inexpensive, but further studies with greater numbers of dogs and more narrow inclusion criteria will be necessary to definitively investigate prolotherapy in dogs.
Footnotes:

a Hi-Rez Versatek Walkway, Tekscan Inc, South Boston, MA 02127, USA.

b Tekscan Pressure Measurement System Walkway Software 7.02, Copyright, Tekscan Inc., South Boston, MA, 02127, USA.

c hydromorphone- Westword, Eatontown, NJ 07724, USA

d acepromazine- Vedco, St. Joseph, MO 64507, USA

e Grafco® two-arm plastic goniometer

f sterile water- Hospira Inc, Lake Forest, IL 60045, USA

g 2% lidocaine- Hospira Inc, Lake Forest, IL 60045, USA

h 50% dextrose- Hospira Inc, Lake Forest, IL 60045, USA

i WINKS 6.0.93, TexaSoft Inc, Cedar Hill, Texas.
Figure Legends

Figure 2.1: Percent Mean Change in Peak Vertical Force- Percentage mean change (box) and standard deviation (whiskers) in Peak Vertical Force (% body weight) from 0-6 Weeks, 6-12 Weeks and 0-12 Weeks.

Figure 2.2: Percent Mean Change in Peak Vertical Impulse- Percentage mean change (box) and standard deviation (whiskers) in Vertical Impulse (% body weight) from 0-6 Weeks, 6-12 Weeks and 0-12 Weeks.
References


Appendix A - Lameness Scoring based on Roush, et al*

Lameness
1 Stands and walks normally
2 Stands normally, with slight lameness at walk
3 Stands normally, with severe lameness at walk
4 Abnormal posture when standing, with severe lameness at walk
5 Reluctant to rise and will not walk > 5 strides