SCINTIGRAPHIC EVALUATION OF COSEQUIN DS AND COSEQUIN DS WITH S-ADENOSYL -L - METHIONINE AS TREATMENTS FOR ACUTE JOINT DISEASE IN DOGS

by

SHERMAN ORYE CANAPP, JR.

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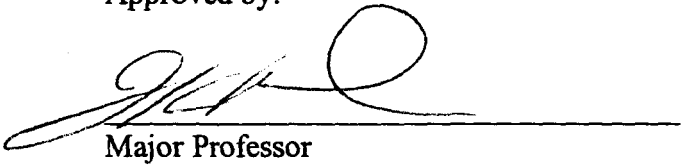

Major Professor
James K. Roush, DVM, MS
Diplomate ACVS

ABSTRACT

Objective—To evaluate the effects of orally administered glucosamine hydrochloride (GlAm)–chondroitin sulfate (CS) and GlAm–CS–S-adenosyl-L-methionine (SAMe) on chemically induced synovitis in the radiocarpal joint of dogs.

Animals—32 adult mixed-breed dogs.

Procedure—For 21 days, all dogs received a sham capsule (3 groups) or GlAm-CS (prior treatment group) in a double-blinded study. Unilateral carpal synovitis then was induced by injecting the right radiocarpal joint with chymopapain, and the left radiocarpal joint (control joint) with saline (0.9% NaCl) solution. Joints were injected on alternate days for 3 injections. After induction of synovitis, 2 groups receiving sham treatment were given GlAm-CS or GlAm-CS–SAMe. The fourth group continued to receive sham capsules (control group). Joint inflammation was quantified, using nuclear scintigraphy (before injection of joints and days 13, 20, 27, 34, 41, and 48 after injection). Lameness evaluations were performed daily.

Results—Dogs given GlAm-CS before induction of synovitis had significantly less scintigraphic activity in the soft-tissue phase 48 days after joint injection, had significantly less uptake in the bone phase 41 and 48 days after joint injection, and had significantly lower lameness scores on days 12 to 19, 23, and 24 after injection, compared with other groups.

Conclusions and Clinical Relevance—Analysis of results of this study suggest that prior treatment with GlAm-CS for 21 days had a protective effect against chemically induced synovitis and associated bone remodeling. Prior treatment with GlAm-CS also reduced lameness in dogs with induced synovitis.

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I. LITERATURE REVIEW

Joint Disease, Part I: A Review of Anatomy, Function, and Pathology

Anatomy of the Canine Antebrachiocarpal Joint:

The antebrachiocarpal joint is a compound ginglymus (hinge) joint that allows flexion and extension with a small amount of lateral angulation.[1] It is comprised of the distal ulna and radius proximally, and the proximal row of carpal bones distally. The radius is concave and angulated craniodistal-caudoproximally 10-15 degrees at the distal articular surface (trochlea).[1] The styloid process is the origin of the radial collateral ligaments. The radius articulates distally with the styloid process of the ulna, which articulates with the accessory and ulnar carpal bones. [1]

Wind reported that the antebrachiocarpal joint will allow 100 degrees of flexion, 10 degrees of hyperextension, 5 degrees of varus and 15 degrees of valgus angulation under normal circumstance. [2] this movement accounts for 80-90 % of the overall carpal movement. [1]

The carpus has but minimal stability and relies on the surrounding soft tissue structures for support. [1] When the dog is standing in its normal posture there is hyperextension due to loading. The palmar ligaments and fibrocartilage pad supply the most support and prevent hyperextension. [1]

The antebrachiocarpal joint also includes articular cartilage, subchondral bone, synovium, joint capsule, and synovial fluid. The joint cavity is defined by a joint capsule that includes the entire joint. [3] The capsule may be further divided into three layers: the synovial lining layer (synovial intima), subsynovial layer, and the fibrous joint capsule.[3] The synovial initima is the innermost layer, is very thin, and is composed of two types of synoviocytes. Type A synoviocytes are involved in removing debris and processing antigens from within the joint. Type A cells are said to be "macrophage-like". Type B cells are said to be "fibroblast-like". [4]The synovial lining does not extend into the articular surface but rather extends from the margin of the articular surface to a point of insertion of the joint capsule.[5] Fibroblasts, loose areolar connective tissue, nerve endings, and a vascular stroma are included into the subsynovial layer. The fibrous layer is the tough outer layer which supplies physical stability to the joint. The fibrous layer is well innervated, vascular, incorporates ligaments, and attaches to the bone close to the articulating surface by a fibrocartilagenous insertion. [5]

The function of the synovium is to selectively prevent large molecules such as proteins from entering the joint cavity. Synovial fluid is a clear dialysate of plasma containing mucin which is produced by the synovial membrane cells which in turn accounts for the high viscosity of joint fluid.[6] Since the joint fluid does not contain clotting factors, it does not clot unless synovial inflammation , or injury as in a traumatic tap has occurred. [6]

Joint fluid allows for the low coefficient of friction between the two cartilage surfaces in the normal articulating joint.[8] Furey has described two forms of joint lubrication, hydrostatic (weeping) lubrication and boundary lubrication.[8] As cartilage is compressed under high loads, hydrostatic lubrication occurs. During this weeping form of lubrication water is squeezed from the viscoelastic gel in the cartilage matrix and accumulates in the depressions on the articular surface.[7] The greater the load applied, the greater the amount of fluid present. Once the loading forces are withdrawn, the fluid is returned to the cartilage matrix. [7] Armstrong has reported that the load required to cause complete contact between joint surfaces is reduced with age, and is likely to reduce the efficiency of "squeeze-film", or weeping lubrication. [9] In addition, the lubricating glycoprotein layer adherent to the articular surface thickens with age, which suggests an attempt to compensate for the increased cartilage compliance by a move from weeping to boundary lubrication. [9] Boundary lubrication however, is the lubrication that occurs between the cartilagenous surfaces during non-loading-bearing motion. The actual lubricant is believed to be a glycoprotein lubricin. Simkin has reported that hydrostatic is not, and boundary minimally, dependent on hyaluronan/hyaluronate concentration. [7]

Normal hyaline cartilage is found at the end of long bones and is an avascular, aneural, and alymphatic tissue which is smooth, resilient, and wear resistant. It allows for nearly frictionless motion and protects subchondral bone from compressive load and shearing forces. [10] Cartilage consists of cells called chondrocytes which synthesize and deposit around themselves an extracellular matrix comprised of water, collagen, and

proteoglycans. [11] Chondrocytes make up only 5% of the over all cartilage volume and are enclosed within a pericellular capsule. The chondrocyte, capsule, and pericellular matrix are together considered the "chondron". [12] Poole has reported that this chondron is capable of responding to changing properities within it's surrounding environment. [11] Collagen is a tough ropy protein that physically connects the tissues, and provides the tensile strength of cartilage. According to Mayne there are 19 different types of collagen. [13] The collagen fibrils are composed of monomers (3 polypeptide "alpha" chains) of protein stacked in a quarter-stagger array. Changes in alpha chain conformations are what constitute the different types of collagen. Williams has reported that types I, II, III, V, XI collagens form fibrils and the remaining do not. [14] Type II collagen is the predominant form in articular cartilage. Poole has also reported that the function of type VI collagen, found in the the pericellular regions, is to help bind the chondrocyte surface to the matrix collagen and proteoglycans. [15] It is speculated that type XI collagen links collagen type II fibrils together and controls their separation by proteoglycan swelling, and possibly binds proteoglycan molecules to collagen type II. [13]

Extracellular matrix that is not collagen is composed of proteoglycans; approximately 22%-38% of articular cartilage dry weight. [16] Stockwell has described the proteoglycan monomer as comprised of a core protein to which one or more types of glycosaminoglycan chains are attached. Chondroitin sulfate, keratan sulfate, and dermatan sulfate are the most common glycosaminoglycans of articular cartilage. Repeating disaccharide subunits (N-acetyl galactosamine, glucuronic acid, N-acetyl

glucosamine, galactose) form chains that make up these glycosaminoglycans. There is an overall negative charge on glycosaminoglycans which causes them to remain separated when attached to a core protein. This negative charge is due to the carboxyl and sulfate groups. Many of the hydrophilic properities of the proteoglycan can be attributed to an osmotic gradient created by the polyanionic nature and excess of molecules in the cartilage matrix versus the external solutions. [17] Sandy and Rosenberg have grouped proteoglycans into two distinct groups, aggregating or nonaggregating, depending on the ability of proteoglycan monomers to aggregate with hyaluronan. Hyaluronan is a non-sulfated glycosaminoglycan that is found in the extracellular matrix and does not bind to a core protein. Aggregates of proteoglycan monomers and hyaluronan that are found in the articular cartilage have been termed aggrecans. These aggrecans are composed of a core protein which are divided up into domains. Sandy and Rosenberg have described these domains as G1 hyaluronan binding region on the amino terminal end, G2 glycosaminoglycan binding region, and G3 carboxy-terminal end. The aggrecan monomer are composed of glycosaminoglycans (Chondroitin and keratan sulfate). The G1 region is composed of aggrecan monomers attached to a hyaluronic acid chain which forms aggrecan aggregates and are stabilized by a link protein. Articular cartilage contains aggrecan aggregates which are made up of over 100 aggrecan monomers.The condition and location of the cartilage determines the length of the aggregan aggregates. [18]

Hyaluronan has been described by Muir as an important component of the articular cartilage matrix and synovial fluid as previously described. It is produced by

both the chondrocytes and type B synoviocytes and is found within the extracellular matrix and synovial fluid respectably. [19] Within the synovial fluid, hyaluronan has been reported to act as a lubricant. In addition, due to its steric conformation it is considered a molecular barrier which allows hyalruonan to block macromolecules from its domain. [17] Hyaluronan is considered a boundary lubricant and though found on the articular cartilage surface layer has no function in cartilage-on-cartilage lubrication. [3] It has also been identified in intra-articular ligaments. [17]

Proteoglycan distribution, chondrocyte organization, and collagen fiber orientation have been described by Poole to be morphologically zonal (Z1-Z4). Zone 1 is superficial and contains sparce cellularity, minimal proteoglycan content, and tangentially oriented fibrils. Zone 2 contains the majority of the matrix volume with an increase in cellularity and proteoglycan content. Zone 3 has yet a further increase in chondrocytes and proteoglycan content with the cells arranged in vertical columns, with the collagen fibrils radially aligned. Zone 4 is composed of calcified cartilage layer which contains radially oriented collagen fibrils but minimal proteoglycan content. Zone 4 is adjacent to the subchondral bone and separated from it by a cement line termed the osteochondral junction. This cement line assists in adhering the cartilage to the subchondral bone. [10]

Aydelotte and Kuettner have reported that the tangentially oriented collagen fibrils of Zone 1, and the low proteoglycan content create an optimal environment for withstanding high tensile stresses and spreading the load of these forces more evenly over

the joint surface. Zone 2 and 3 contain a much higher content of proteoglycans allowing an even greater ability to withstand compressive loads. Zone 4 changes from the more compliant nonmineralized cartilage to a less compliant stiffer calcified cartilage.[20] The osteochondral junction (cement line) is not held together by collagen fibils, but rather undulating, interdigitating contours. This structural alignment allows shear stresses to be converted so that destructive compressive forces on the underlying subchondral bone are minimal. [21]

Kuttner has reported that proteoglycans normally contained within their meshwork of collage fibrils have an extremely high affinity for water. It is the collagen network that limits the amount of water, and therefore swelling that the proteoglycans can undergo.The swelling that does occur allows the cartilage to remain turgid which decreases deformation due to compressive forces. When compressive forces occur water is allowed to move through pores created by proteoglycan molecules within the cartilage matrix.[22] There is a certain amount of water that is forced out while under compressive forces. This is termed weeping or hydrostatic lubrication as previously described.

Beneath Zone 4 is a thin plate of bone that is in direct contact with the calcified layer of cartilage and the cancellous bone supporting this bony plate, this is considered the subchondral region. There is a lattice-like meshwork at the epiphyseal end of bone known as cancellous bone. Radin has reported this type of bone to be 10 times more deformable that cortical bone. This deformability plays a crucial role in compressive

force distribution. It is the responsibility of the subchondral bone, due to its compliance, to reduce peak load and decrease the potential damage to cartilage. [23]

PATHOLOGY OF THE JOINT

Degenerative joint disease (DJD) also known as osteoarthritis (OA), is a debilitating disorder affecting a wide range of animal species and humans. It is a syndrome that affects all the components of synovial joints including the articular cartilage, subchondral bone, synovium, joint capsule, and synovial fluid. Evidence of DJD has been found in the fossils of prehistoric dinosaurs as well as in the joints of ancient mummies. [24] It is unfortunate however, that despite the long existence of this disease, the facts concerning its etiology and factors triggering its development are still incompletely known. [25] Bennett has described osteoarthrosis as being not only one of the earliest diseases, but one of the most frequently encountered joint disease found in dogs. [26] There are many causes of the disease, but because the joint, like any other tissue, can only respond to injury in a limited number of ways, the final pathway is osteoarthrosis. This disease process is said to be irreversible, but at what stage in development changes become irreversible is still unknown. [27]

There are multiple terminology's used to describe the various forms of joint disease. These include Degenerative Joint Disease (DJD), Osteoarthritis (OA), Osteoarthrosis, and Secondary Joint Disease. According to May the term DJD neglects to

consider the increased anabolic state of the cartilage, in addition to the lack of progression that may be accompanied. May has also found the term "osteoarthritis" to be somewhat inconsistent due to the fact that synovitis is often minimal. Osteoarthrosis may be a preferred term indicating a pathological process rather than an acute inflammatory process. [28]

Todhunter has described osteoarthritis as a degenerative disease of synovial joints characterized by joint pain and disability, loss of articular cartilage (fibrillation, ulceration, and finally eburnation), and bony remodeling. [27] There have been two classifications of osteoarthritis reported by Prostredny. Primary (idiopathic) osteoarthritis is found in locations that have not been induced by trauma or disease. Primary osteoarthritis is rare in dogs. Secondary osteoarthritis results from multiple factors. These factors include: trauma (fractures, ligamentous damage), inflammation (infectious and immune-mediated), congenital/developmental (osteochondritis dissecans and hip dysplasia), metabolic, endocrine, neuropathic, neoplastic, or iatrogenic (drug reactions). [29]

Whether abnormal stresses are being placed on normal cartilage or normal stresses placed on abnormal cartilage, osteoarthritis is still the outcome. The net effect is cartilage loss (loss of proteoglycan, disruption of collagen network), subchondral bony sclerosis, synovial membrane inflammation, enthesiophytosis, the development of periarticular osteophytes, and joint instability. [29]

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Todhunter states that there are two theories on the etiopathogenesis of osteoarthritis (OA); the first is that the primary event occurs in the articular cartilage and all other events are secondary; the second suggests that cartilage degeneration is secondary to synovitis or subchondral bony changes. [27]

Though the etiology of OA varies, inflammation of the synovial membranes and joint capsule are usually one of the results. It is not common for cartilage damage to be present at this early stage. The inflamed synovial membrane (synovitis) allows leukocytes to invade the joint space. Both the synovial cells and leukocytes release destructive enzymes (free radicals, cytokines, and prostaglandins) into the joint cavity which further inflame the synovium and modulate the metabolism, damaging the articular cartilage matrix. [30] Synovitis is responsible for a majority of the pain found in the osteoarthitic
joint. [27]

Fibrillation (flaking of the superficial cartilage layers) is one of the first microscopic changes noted with OA. This lesion appears as a microscopic roughening of the articular surface due to separation between collagen fibrils that run parallel to the joint surface. [20] The stiff outer layer then loses its integrity and exposes the underlying cartilage to abnormal stress. These stresses lead to the development of fissures in deeper layers which due to their vertical alignment, extend to the level of subchondral bone.[20]

Chondrocytes and cartilage matrix show distinct morphology changes as part of the degenerative process. The normal distribution of chondrocytes is altered into clusters and the overall size of the cells increases. Thinning of the cartilage occurs in areas of direct contact, while thickening actually occurs in areas of indirect contact. These events may lead to complete loss of cartilage followed by exposure of subchondral bone. Cartilage fragments are released from the degraded cartilage into the synovial fluid. These fragments may migrate to the synovium where they set up an inflammatory response, and are phagocytized by type A synoviocytes. [31]

One of the main biochemical change that occurs in cartilage is the loss of proteoglycans. The amount lost varies with the severity of disease and according to Mankin, occurs while the collagen content remains normal. Loss of proteoglycans takes place despite an increase in its synthesis. [32]

Increasing water content within the cartilage is an early change found in OA. Degradative proteases released from damaged chondrocytes break down collagen cross-links, resulting in abnormal hydration of the proteoglycans, greater separation of fibrils, resulting in an increased thickness of articular cartilage. According to Broom this process results in collagen fibrils aligning radially, disrupting the interlocking meshwork, which decreases the ability to retain proteoglycans and resist compressive forces. Proteoglycans are released into synovial fluid, and articular cartilage is even more susceptible to mechanical injury because of its softer than normal composition. [33]

The relationship between cartilage degeneration and osteophyte formation remains unclear. In a study performed by Gilbertson, after experimental sectioning of the cranial cruciate ligament in dogs, osteophyte formation was noted as early as three days post-op.[34] Trueta suggests that osteophytes develop as a result of blood vessel proliferation in regions of cartilage degeneration.[35] There is also increased subchondral bone pressure in association with OA, resulting from subchondral bone remodeling. In addition, peri-articular osteophytes have been found proceeded by thickened articular cartilage adjacent to the synovial membrane. This suggests that peripheral cells were stimulated to undergo endochondral ossification. [36]

The proteases play a key role in the degradation of structural proteins of the extracellular matrix. Under normal conditions chondrocytes use these enzymes to remove existing matrix as part of normal turnover.[37] Barrett has classified proteinases into four categories on the basis of the chemical groups which determine their activity: (1) aspartic proteinases, (2) cysteine proteinases, (3) serine proteinases, (4) metalloproteinases. [39,39] These proteases may be found in chondrocytes, synoviocytes, and inflammatory cells. The breakdown of articular cartilage is thought to occur mainly by the action of metalloproteinases of which there are 11 different molecules. [37] Collagenases , stromelysins, and gelatinases are thought to be the most significant in OA and may act on multiple substrates. Under normal conditions metalloproteinase inhibitors control

cartilage catabolism, however, in OA there is an imbalance between the inhibitors and the proteases. [37]

Injured chondrocytes may release cytokines either concurrently or prior to their release of proteases according to Lotz. Interleukin-1(IL-1), Interleukin-6, and tumor necrosis factor alpha are thought to be the most important in regards to OA. These cytokines further stimulate chondrocytes and synoviocytes to produce and release more degradative enzymes. [40] IL-1 has been shown to stimulate fibroblasts to produce collagen types I and III which is thought to contribute to the fibrosis of the joint capsule in OA. [41]

While the etiopathogenesis of OA remains unclear, the interaction of biochemical and biomechanical factors perpetuating degenerative changes seems to be unanimous. If an initial biochemical response is elicited, it is likely that mechanical factors result in destruction of the cartilage matrix in symphony with the enzymatic degradation. Should biomechanical factors initiate the response, biochemical degradation parallels.

EVALUATIONS

It is obvious that in order to determine the severity or progression of joint disease in an animal a thorough clinical evaluation, followed by the appropriate diagnostic procedures should be performed. Radiography, scintigraphy, and lameness examinations were the diagnostic techniques utilized in this study. Other diagnostic procedures that may be used for the study of joint disease include: Diagnostic imaging (microfocal radiography, contrast radiography, xeroradiography, linear tomography, computerized

tomography, magnetic resonance imaging, and ultrasonography), arthrocentesis, forceplate and kinematic gait analysis, surgical exploration (arthroscopy, arthrotomy) , and synovial biopsy.

Radiography (survey films) are the most commonly used diagnostic aid used to establish the diagnosis of osteoarthritis, to determine the severity and extent of the disease, and to assess the progression of disease. [42] Conventional radiography shows damage to the cartilage indirectly through joint space narrowing and damage to bone in the form of osteophytes and subchondral sclerosis. [43] Houlton has described the possible radiographic changes associated with joint disease to include: distention of the joint capsule, abnormal relationship of bones, altered width of joint space, calcified articular cartilage, articular fractures, intra-articular joint mice, subchondral bone changes (erosions, sclerosis, and cysts), calcification of peri-articular and intra-articular soft tissues, peri-articular osteophytes, and peri-articular enthesophytes. [44] Radiological assessment on survey radiographs has many advantages for measuring the long term out-come of OA. First, radiographs are inexpensive, widely available, and do not require special facilities. Radiographs form a permanent record, and can be assessed by the same reader regardless of the time or location in which they were taken. [45] However, there are many disadvantages to survey radiographs as well. The progression of radiographic OA is slow and the sources of variability in the measurement process are numerous (joint positioning, radiographic procedure, measurement error due to reader or instrument). Adams and Wallace have reported that thinning and defects in the surface of articular

cartilage, changes diagnostic of osteoarthritis, are not visible on survey radiographs. Narrowing of the joint space has been used to identify loss of articular cartilage in man, however, the impracticality of obtaining radiographs during weight-bearing prohibit measurement of the thickness of articular cartilage in dogs. [42]

Nuclear scintigraphy is an excellent method for quantifying inflammation and bone activity within a joint. Scintigraphy is able to evaluate the activity of bone by measuring the uptake of an intravenously administered radiopharmaceutical isotope. [44] The radiopharmaceutical isotope used most commonly is ^{99m} Technetium methylene diphosphonate complex. The actual mechanism of action is still not completely understood. Jones stated that it is thought that phosphate compounds chemisorb (absorb by chemical bonding) to amorphous bone (metabolically active) and at kink and dislocation sites on the surface of the hydroxyapatite crystal, with the release of the tin reducing agent as well as the bound ^{99m}Tc label. The latter are then hydrolyzed and bound to bone, either separately or together as hydrated tin oxide and technetium dioxide.[46]

Siegel has shown that tracer uptake is proportional to regional blood flow, as evidenced by increased tracer bound at sites of inflammation and in reflex sympathetic dystrophy. [47] The half-time clearance from the vascular to the extravascular space is 2 to 4 minutes post intravenous injection. Within 3 hours 30 to 40% of the injected dose is bound to bone, approximately 35% has been excreted by the kidneys, 10 to 15% is associated with other tissues and 5 to 10% remains in the blood. [48] There are three

clinically significant phases that occur as the tracer moves through tissues over time. Phase 1: the majority of the tracer is intravascular (vascular phase). Phase 2: the tracer is both intravascular and extravascularly distributed throughout the extravascular cellular fluid (1 to 2 min.) (soft tissue phase). Phase 3: the tracer is mostly associated with bone (bone phase). [48] The maximum bone uptake for 99mTc-MDP occurs at about 65 minutes in well hydrated animals, whereas the optimum contrast, or target-to-background, ratio occurs at about 6 hours according to Makler. [49] The rate of bone turnover and blood supply also determine the quantity and distribution of tracer uptake by bone. Goergen has reported that if there is a reduction of blood flow to a region of bone, as in osteonecrosis or radiation injury, a photon-deficient, or "cold" lesion will result. If there is an increased blood flow (hyperemia) to a region of bone, increased bone uptake occurs. [50]

Because scintigraphy is so sensitive, it may be useful in establishing the presence of arthritis when radiographs are normal. Scintigraphy is also useful in judging the severity of disease. [51] Harbert has reported that the rationale for using bone tracers in joint imaging was that joint diseases are frequently associated with new bone formation, presumably because of the increased synovial vascularity.[51] The disadvantages of scintigraphy is that it is nonspecific,[42] lacks spatial resolution, is expensive, and entails many precautions for its safe use. [44]

Subjective lameness grading can be broken into two types: Numerical rating scale (NRS) and a visual analog scale (VAS).[52] The NRS consists of numerical divisions, or grades which range in scale according to author. The classifications range according to author from the lowest number being sound (ex. Grade 1) to the highest being completely non-weight bearing (ex Grade 5).

The VAS utilizes a 100-mm horizontal line with vertical bars at either end, one end labeled "sound" and the other labeled "non-weight bearing". A mark is then subjectively placed along the axis according to the animals lameness. Studies in which two independent observers graded lameness using VAS or NAS indicated no significant difference between the 2 observers scoring lameness, using either the VAS or the NRS. In these studies the VAS and NRS scores for the observer were highly reproducible. The studies concluded that although the NRS and VAS compared favorably with respect to repeatability, reproducibility, and use by 2 observers, the VAS was inherently more sensitive. In addition, the NRS and VAS should not be used interchangeably.[52,53]

MEDICAL MANAGEMENT & TREATMENT

Slow-acting, disease-modifying osteoarthritis agents

Joint injury and subsequent OA occur commonly in man and animals. It has been reported that OA may affect up to 20% of the canine population over one year of age. Johnson has reported in a recent study that nearly 50% of musculoskeletal disorders

identified over the last 10 years in 16 veterinary hospitals were due to joint disease.[54] Unfortunately, therapy for joint disease has found limited success once the bony changes associated with degeneration have developed. Preventing or minimizing the inflammatory process within the joint (synovitis) can interrupt the cascade of events that lead to cartilage degeneration. Controlling or preventing synovitis early in the disease process may slow the progression of degenerative joint disease. Currently, the ideal treatment for synovitis has not been is found, though the identification of such treatment is essential if the disease is to be controlled before irreversible osteoarthritis occurs.[55]

Traditional therapies such as corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been used in the past for the treatment of OA. However, Manley has stated that these agents produce undesirable side effects that can occur with long term use, and these agents have deleterious effects on chondrocyte and matrix homeostasis.[56] Gonzalez suggest that the apparent deleterious impact of some NSAIDs on DJD progression may be due to their inhibitory activity on the synthesis of prostaglandins.[57] Unfortunately, many of these compounds contribute to generation of catabolic cytokines (cause chondrocytes to degrade matrix), or prevent expression of anabolic cytokines, with a resulting downward spiral and progression of OA.[58]

Attention has now shifted to alternative modes of therapy for the medical management of OA with research focusing on slowing the process of cartilage degradation and promotion of cartilage matrix synthesis.[59] This research has led to the

development of a new class of products termed "slow-acting, disease-modifying osteoarthritis agents".[60] These products can be further divided into parenterally administered products (PSGAG; Adequan;Luitpold Pharmaceuticals, Shirley, NY), and orally administered products (Glucosamine hydrochloride and chondroitin sulfate;Gylcoflex;Vetri-Science Laboratories, Essex Junction, VT, and Cosequin;Nutramax Laboratories, Baltimore, MD) which act completely different from analgesics.[60] These compounds were previously termed chondroprotective agents since they directly affect the chondrocytes in a beneficial manner.[58] Bassleer has reported that these agents counter the destructive inflammatory process and encourage normalization of the synovial fluid and cartilage matrix.[61] Ghosh and Smith have defined chondroprotective agents as compounds that: (1) Support or enhance macromolecular synthesis by chondrocytes (DNA, RNA, collagen, proteoglycans, matrix), (2) support or enhance synthesis of hyaluronan by synoviocytes in synovial fluid, (3) inhibit degradative enzymes (e.g., collagenase, cathepsins, hyaluronidase, chondroitonases) or inflammatory mediators (interleukin –1, tumor necrosis factor, PGE2), and (4) remove or prevent formation of fibrin, thrombi, or plaque in synovium and/or subchondral blood vessels.[62] At the present time, no know drug is capable of performing all of these objectives.

According to Bucci the compounds capable of these actions are identical to the compounds used by the body to manufacture proteoglycans (PGs), specifically, glycosaminoglycans (GAGs).[63] The following is a list of GAGs and their derivatives that have been studied clinically for OA: Glucosamine salts, Chondroitin sulfate (CS),

Hyaluronan, Arteparon (synthetic CS), Rumalon (PGs extracted from bovine cartilage), Pentosan Polysulfate (synthetic GAGs) and S-adenosyl-L-methionine. Pentosan polysulfate and S-adenosyl-L-methionine are the only agents listed that do not naturally occur in normal cartilage and connective tissue. Arteparon and Rumalon are administered by injection and therefore considered a drug by FDA's standards.[63] This leaves glucosamine salts, chondroitin sulfate, and S-adenosyl-L-methionine as the only natural agents, administered orally as slow-acting, disease-modifying osteoarthritis agents.

Glucosamine (GlAm) and Chondroitin sulfate (CS) in either purified, extracted, or complexed form are the main compounds in the majority of orally slow-acting, disease-modifying osteoarthritis agents. The two most commonly used oral supplements containing these compounds are Cosequin (Nutramax Laboratories, Baltimore, MD), and Glycoflex (Vetri-Science Laboratories, Essex Junction, VT). Cosequin contains purified glucosamine, CS, and manganese ascorbate. Glycoflex is an extract from the Perna canaliculus mollusc exoskeleton, which is reported to contain glucosamine and CS.[60]

Glucosamine

Glucosamine (GlAm) is an amino-monosaccharide nutrient (molecular wt, 179).[61] GlAm is soluble in water and has a pKa of 6.91 at 37°C. Pharmokinetic studies on GlAm in man and dogs using ^{14}C-glucosamine have indicated that 54% of GlAm is nonionized

in the small intestine (intestinal pH of 6.8), however, most of the GIAm is ionized in the acid of the stomach.[64,65] A favorable environment for absorption is in the small intestine, not the stomach. GIAm is absorbed easily and distributed to cartilage, where they have been shown to be incorporated into the cartilage matrix. Setnikar has reported that the physiochemical properties of GIAm account for a favorable pharmacokinetic profile including oral bioavailability and specific cartilage tropism, as was shown in animals and humans using radiolabeled compounds. Excretion is primarily via urine and feces, with 87% of orally administered GIAm being absorbed.[64,65] The basic mechanism of action of GIAm is to provide the regulatory stimulus and raw materials for synthesis of GAGs. In a study by Setnikar, glucosamine exhibited no toxicity at high oral doses.[66]

Bassleer has described glucosamine as a precursor of the disaccharide unit of GAGs, which is the building block of the ground substance of the articular cartilage (proteoglycans).[61] Chondrocytes obtain preformed glucosamine from the circulation, or synthesizes it from glucose and amino acids. Glucosamine levels are critical to subsequent macromolecular synthesis regardless of the source. Burkhardt indicated through biochemical and pharmacological studies that administration of GIAm tends to normalize cartilage metabolism and stimulate the synthesis of proteoglycans so that articular function is partially restored.[61] GIAm is used directly for synthesis of hyaluronic acid by synoviocytes, and for all other GAGs after conversion to amino sugars by epimerases. This shows that GAIm is more that just a building block for GAGs, it is also an up-regulator of GAG synthesis (cartilage matrix synthesis). Bucci has reported that

GlAm stimulates GAG, PG, and collagen synthesis in chondrocytes and fibroblasts.[63] Karzel has demonstrated that both glucosamine HCL and glucosamine sulfate were equally effective in stimulating glycosaminoglycans in cell cultures.[67]

In a study performed by Jimenez, the effects of glucosamine sulfate (GlAm) on human chondrocyte gene expression was evaluated. The effects of type II collagen, fibronectin, stromelysin and the proteoglycans, aggrecan, perlecan and biglycan by normal adult articular cartilage chondrocytes were examined. Results of this study showed that GlAm affected chondrocyte biology by its ability to modulate the expression of the important cartilage proteoglycans. Results also showed that GlAm causes a modest decrease in the levels of stromelysin mRNA in OA chondrocytes and that it does not adversly affect the constitutive expression of type II collagen or fibronectin in normal or OA chondrocytes.[68]

There have been many human clinical trials that have evaluated the effects of glucosamine sulfate on OA. Crolle performed a study in which injectable (intra-articular or intramuscular) followed by oral form of glucosamine sulfate were investigated in patients with osteoarthrosis. Semi-quantitative scoring of pain at rest and during passive movements, of restricted function and, where possible, of walking time over 20 meters, were taken as therapeutic activity indices. It was found that with both parenteral treatments, each symptom significantly improved when compared to placebo.[69] Drovanti evaluated oral glucosamine sulfate in 80 patients with osteoarthrosis. Articular pain, joint

tenderness and swelling, and restriction of active and passive movements were scored. In addition, samples of articular cartilage were analyzed by scanning microscopy after the end of treatment. Drovanti found that patients treated with glucosamine sulfate experienced a reduction in overall symptoms that was almost twice a large and twice as fast as those who had placebo. Electron microscopy also showed that those who had glucosamine sulfate showed a picture more similar to healthy cartilage than those who had placebo.[70] Noack also conducted a double-blind, randomized, placebo-controlled study evaluating the effects of glucosamine sulfate in OA of the knee of 252 patients. His study was able to show that oral GIAm was significantly more effective than placebo in improving pain and movement limitation in patients with OA using the Lequesne's criteria.[71] In a study by Muller-FaBender GIAm was compared to ibuprofen in OA of knee. This randomized, double-blind, parallel-group study included 200 hospital patients with active OA of the knee, symptoms for at least 3 months and a Lequsne's index of at least 7 points. This 200 patient comparative 4-week study demonstrated that GIAm was as effective as ibuprofen in controlling symptoms in patients with active OA. According to Muller-FaBender, GIAm was better tolerated than ibuprofen.[72]

GIAm has not been shown to inhibit the synthesis of prostaglandins and has no known toxicity at high dose levels.[66]

The use of parentral polysulfated glycosaminoglycans (PSGAG) has been evaluated for the treatment of OA. In a study performed by Lust, the effects of

intramuscular administration of glycosaminoglycan polysulfates was evaluated in growing pups with hip dysplasia. Lust found that IM administration of glycosaminoglycan polysulfates from 6 weeks to 8 months of age in growing pups that were susceptible to hip dysplasia resulted in less subluxation as determined through radiography. Treated pups also had closer coxofemoral congrunity at 8 months of age. The joint pathologic scores at necropsy in the treated pups indicated a trend toward improvement (more normal synovial fluid volume and lower ligament volume) but the differences were not significant.[73] DeHann also evaluated the effects of intramuscular polysulfated glycosaminoglycan (PSGAG) for the treatment of hip dysplasia in 84 dogs. In his study response to treatment was analyzed based on changes in lameness, range of motion (ROM), and pain on manipulation of the hip joints. DeHann found that dogs administered PSGAG showed the greatest improvement in orthopedic scores compared to placebo; however, the differences in clinical improvement were not statistically significant. In his study, no local or systemic adverse reactions related to the drug were observed.[74] In a study by Hannan, the systemic administration of PSGAG (Arteparon) provided partial protection of articular cartilage from damage produced by meniscetomy in the canine. This protection was demonstrated histologically by reduced surface fibrillation, diminished chondrocyte cloning, and maintenance of alcianophilia.[75]

PSGAG has also been evaluated in the equine for the treatment of OA. In a study by Yovich, the effects of intra-articular PSGAG on repair of chemical and physical articular cartilage injuries was evaluated in 8 horses. Results of his study demonstrated

that PSGAG had chondroprotective properties in a model of chemically induced articular cartilage damage, whereas PSGAG had no effect in a pyhsical articular cartilage-defect model.[76] Todhunter performed study evaluating the effects of PSGAG in 18 ponies. Combined blood pool and delayed images produced by the use of 99mTcMDP were used to objectively evaluate the response of equine joints with osteochondral defects to postoperative exercise and intra-articularly administered PSGAG. Results of Todhunter's study showed he combination of exercise and medication in individual joints resulted in a significant decreased bone remodeling.[77]

Chondroitin Sulfate

Chondroitin sulfate (CS) is a long chain polymer of a repeating disaccharide unit (galactosamine sulfate and glucuronic acid), (molecular wt. 10kd).[78] It is the predominant GAG found in articular cartilage and is a natural component of several other body tissues (tendons, bone, vertebral discs, heart, and cornea).[78] CS has been purified from bovine, whale, and shark cartilage sources and has been studied extensively in humans as a successful anti-atherosclerotic agent.[79] Bucci has reported that CS differ from GAIm in that CS stimulate GAG and PG synthesis by extracellular as well as intracellular mechanisms. CS competitively inhibits degradative enzymes of PG in cartilage and synovial through its long chain length.[63,80]

Bioavailibilty studies on humans and experimental animals have shown 70% absorption following oral administration.[81,82] Absorption studies of CS has been reported in the rat, dog, and human, and have shown significant increases in serum levels of ^{35}S-labeled CS after oral administration of ^{35}S-labeled CS. The CS that was detected in blood had a high molecular weight suggesting that some CS is absorbed intact.[82] Conte has shown a 70% absorption rate based on fecal excretion tests when tritiated CS was administered orally to dogs. Conte has also reported that CS has an affinity for synovial fluid and articular cartilage.[82]

The FDA has ruled that since each CS molecule is slightly different (chain length, number of sulfates per chain, incidence of other monomers), that could not be classified as a single drug because it did not meet the rigid batch-to batch identity.[63]

Bassleer has reported in a study evaluating the in vitro effects of Chondroitin sulfate (CS) on human articular chondrocytes cultivated in clusters. In his study articular chondrocytes were cultivated in the presence or absence of CS. Results of the study showed a significant increase in PG amounts in chondrocyte clusters. CS also induced a significant and dose-dependent decrease of collagenolytic activity released by human chondrocytes. These in vitro results suggest that CS is able to increase PG amounts in the matrix surrounding the cells and to decrease catabolic activities of human articular chondrocytes.[83]

The protective effects of CS was also examined in a study by Uebelhart using an animal model of chymopapain-induced articular cartilage injury. In this study, rabbits

received an intra-articular injection of 2.0 mg chymopapain into the left knee and were sacrificed after 84 days. The contralateral right knee served as a non-injected control. Animals received daily intra-muscular injection or oral doses of CS beginning 10 days prior to the chymopapain injections and then for another 21 days after which treatment was ceased. Results of this study showed that cartilage PG content was reduced less in both intramuscular and oral CS groups compared to control.[84]

There have been many randomized, double-blinded, controlled clinical trials performed on CS and they have shown to be effective in reducing the symptoms of DJD (reducing pain, improved joint function, and mobilization).[85,86] In a study performed by Mazieres, 120 patients with osteoarthritis of the knees and hips were placed in a randomized, placebo-controlled, double-blind trial designed to evaluate the effectiveness of Chondroitin sulfate (CS). At completion of the treatment phase, parameters studied, including a visual analog pain-scale assessment, a pain-function index and overall patient and physician assessments showed a significant trend towards improvement.[86] Uebelhart evaluated the effects of CS on knee OA in 42 patients in a randomized, double-blind, controlled study. Results showed that CS was well-tolerated and significantly reduced pain due to OA, while simultaneously increasing overall mobility of the patients. The metabolism of bone and joint assessed by various biochemical markers also stabilized in the CS patients whereas it was still abnormal in the placebo patients.[87]

Ronca performed a study in which the pharmacokinetics of CS were investigated in rats and in healthy volunteers using CS titrated at the reducing end and labeled with ^{131}I

or ^{99m}Tc respectively. The CS showed a tropism for cartilagenous tissues in rats and for knee tissues in humans as demonstrated by scintigraphic analysis with ^{99m}tC-CS. In synovial fluid of patients requiring joint aspiration, treated orally for 10 days with CS, the hyaluronate concentration and the intrinsic viscosity significantly increased, while collagenolytic activity, phospholipase A2 and N-acetylglucosaminidase (NAG) decreased.[88]

Another compound that is often used with glucosamine and chondroitin sulfate is manganese. Manganese is a trace element and a cofactor in the biosynthesis of GAGs.[89] Specifically, the reactions that make glycosaminoglycans out of glucosamine will not occur efficiently unless manganese is present in the body.[89] Ascorbate (produced naturally in dog's liver) is another compound that is often used with the above combinations. Ascorbate is a reducing agent for the prolyl hydroxylase and lysyl hydroxylase, which catalyze the formation of hydroxyproline and hydroxylysine residues, respectively, in collagen. The fibril formation and cross-linking in articular cartilage utilize these residues.[89]

Together glucosamine salts and chondroitin sulfates meet the definition of oral, slow-acting, disease-modifying osteoarthritis agents. Combining these two agents seem to have a synergistic effect because of the low molecular weight of GlAm and high molecular weight of CS.[63] These agents work synergistically in forming GAGs, inhibiting degradative enzymes, and upregulating cartilage metabolism and matrix production.[63]

Previous studies have shown a synergistic effect from the combined use of GIAm and CS for the treatment of OA. Hulse evaluated the effects of Cosequin in cranial cruciate deficient and reconstructed stifle joints in dogs. In this study 16 dogs (four groups) had their right or left stifle randomly chosen to undergo transection of the cranial cruciate ligament (Pond-Nuki Model). Two groups (I and II) had the injury surgically repaired and two groups (III and IV) served as a non-reconstructed positive control. Following transection of the CCL dogs in groups II and IV were administered 3 Cosequin capsules PO BID until euthanasia (5 months post-op). Results of this study showed that dogs receiving Cosequin subjectively had less intense osteoarthritis than non-Cosequin dogs. In dogs receiving Cosequin, the translations following graft transection more closely resembled translations in the non-operated control joint when compared to dogs not receiving Cosequin. The difference in cranial translation was significant. In addition, the mean modified Mankin score in dogs receiving Cosequin was 50% lower than those not receiving Cosequin.[90]

The effects of an oral chondroprotective agent (Cosequin) on cartilage metabolism and canine serum was evaluated in a study by McNamara. In this study the effects of Cosequin were evaluated by monitoring serum samples drawn from 10 normal dogs at time 0 and after 30 days of daily dosing. Serum aliquots were tested for circulating GAG content by the DMB dye-binding assay. In this study serum was tested for effects on normal cartilage metabolism by culturing normal calf cartilage segments. Results from

this study showed that median serum GAG levels increased significantly. In addition, the biosynthetic activity of chondrocytes was significantly increased. Proteolytic degradation of cartilage segments cultured in serum was reduced with borderline statistical significance. This study suggests that Cosequin, either acting directly on cartilage or by virtue of induction of a factor circulating in serum, reduces breakdown of cartilage matrix concomitant with acceleration of matrix systhesis.[91] In a similar study performed by Lippiello, cartilage stimulatory and antiproteolytic activity were evaluated using sera of dogs treated with Cosequin. 30 dogs were given Cosequin for 30 days after which blood samples were taken and the biosynthetic and degradative responses of bovine cartilage exposed to canine serum were monitored with radiolabeling methods. Results of this study demonstrated a significant elevation in serum GAG levels at 30 days increased by 42%, compared to pre-administration levels. Cartilage segments cultured in canine serum had a 50% increase in GAG biosynthesis. In addition, median proteolytic degradation was reduced by 59%.[92]

The effects of Cosequin on OA has also been evaluated in the equine. Hanson evaluated the effects of Cosequin on 25 horses with DJD. In his study lameness grade, flexion test grade and stride length were measured at initial examination and re-evaluated at 2, 4, and 6 weeks follow-up. Hanson found that within 2 weeks from the start of administration of Cosequin, the lameness grade, flexion test, and stride length were significantly improved. A further significant improvement in lameness was evident at 4 weeks, while flexion score and stride length did not show further improvement.[93] While

there was a significant improvement in the horses treated with Cosequin, irrespective of age, joint affected or use of the horse, it is important to note that this study lacked a placebo group. This factor limits the interpretation of the magnitude of the improvement of the horses. In addition, examiners in this study since were not blinded as to group treatment. Examiner bias may have played a role in the assessment of the outcomes measued.

Hanson has also reported a double-blind, placebo-controlled randomized clinical study evaluating the efficacy of Cosequin in the treatment of navicular syndrome. In this study 10 horses with progressive forelimb lameness and diagnosed with navicular syndrome were assessed by both veterinary specialist and owners. Horses were administered Cosequin twice a day orally for 56 days. A significant improvement was observed in the intervention group in comparison with the placebo group. Significant improvements were discovered in: overall condition; hoof tester examination; total lameness score. No side effects were noted by the specialist or owner in this study.[34]

Oral, slow-acting, disease-modifying agents are widely used for the treatment of DJD despite the fact that little is known about their safety. McNamara performed such a study, evaluating the effects of Cosequin on hematologic, hemostatic, and biochemical variables in clinically normal dogs administered the agent for 30 days. In this study, variables were assessed prior to treatment and on days 3, 14, and 30 of treatment. Results showed significant decreases in hematocrit, hemoglobin, WBC, and segmented

neutrophil variables on days 3 and 14 of treatment. A significant decrease in red cell distribution width was noted on days 3 and 30, in RBC count on day 3, and in lymphocyte numbers on day 30. While there was a significant decrease in platelet count on days 14 and 30, no changes were noted in prothrombin time, activated partial thromboplastin time, mucosal bleeding time, or biochemical variables during the study. Results of this study conclude that administration of Cosequin causes minor, but not clinically significant changes in hematologic and hemostatic variables in young, clinically normal dogs.[95]

In addition to the studies reported above, there have been numerous anecdotal reports claiming improvement in mobility and pain reduction in dogs receiving oral, disease-modifying OA agents. One such report is from Anderson looking at results of a survey of small animal practitioners on the perceived efficacy and safety of Cosequin. In this study a survey was randomly sent to veterinary practices (n = 1540) in the United States who were regular users of the agent. 82% of the practitioners selected responded. Joints treated as the cause of lameness included: shoulder 1,952; elbow 2,398; stifle 5,180; and hip15,261. The percent of practitioners that rated the clinical efficacy of the compound to be "good" or "excellent" in regards to improved mobility, alleviating pain, and improved attitude in animals were 89%, 83%, and 85%, respectively. Radiographic evidence of DJD being treated by the practitioners included: mild DJD 25%; moderate DJD 34%; severe DJD 6%; 38% reported all grades of DJD. The most common reported side effect observed was gastrointestinal upset in 2% of the cases. Results of this study

suggest that based on practitioners experience, Cosequin is both safe and clinically effective.[96]

Moore has published a paper on case summaries for three patients with various forms of osteoarthritis, all of whom received significant benefit from Cosequin therapy. According to Moore, more than three-fourths of the animals treated in his hospital with Cosequin have experienced positive effects, ranging from reduced dependence on anti-inflammatory drugs to dramatic increases in mobility with increases in muscle mass. None experienced adverse effects from Cosequin treatment.[97]

Cosamin, the human version of Cosequin was evaluated in a study to determine if it has an effect on the incidence, severity, immunological response, histopathology or pathogenesis of disease in collagen-induced arthritis (CIA). In this study performed by Beren, animals were administered Cosamin orally for 10 days prior to the initial immunizations with type II collagen and continued until sacrificed at day 42. Results of this study showed that the incidence of arthritis in the control group was 96.5%, while the incidence in the rats receiving Cosamin was significantly different with 54% affected.[98] Cosamin DS was elvaluated in a study by Woodward and Lippiello by monitoring the progression of cartilage lesions in a rabbit instability model of osteoarthrosis. Histologically, using the modified Mankin grading system, rabbits in the control group showed more severe cartilage lesions compared to rabbits in the Cosamin DS group.[99]

S-Adenosyl-L-methionine

S-Adenosyl-L-methionine (SAMe) is a physiological molecule that is present in living cells and participates in a number of intermediate metabolic reactions.[100] SAMe functions in a wide variety of anabolic and catobolic biologic reactions. SAMe is metabolized into homocysteine, then cysteine, and glutathione. It is the glutathione that takes part in the inflammatory process as a scavenger of oxygen-redued products.[100] SAMe, due to its properties, has been found to be a valuable therapeutic tool in the management of neurologic disorders as an antidepressant.[101,102] SAMe treatment is associated with changes in SAMe levels in cerebrospinal fluid and with changes in monoamines, particularly with a marked incrase in CSF 5-hydroxyindoleacetic acid (serotonin metabolism).[102] SAMe has also been utilized in the treatment of liver diseases as detoxicating[103] and cytoprotective agent.[104] In particular SAMe has proved effective in ameliorating intrahepatic cholestasis.[105]

SAMe ia also a precursor of polyamines, which are involved in cell proliferation and protein synthesis. Polyamines are thought to also stabilize the polyanionic macromolecules of proteoglycans, thus according to Conroy, potentially protecting them from destruction of proteolytic and glycolytic enzymes.[106] Polyamines possess anti-inflammatory and analgesic properties and are scavengers of free radicals.[107,108,109]

Studies have shown SAMe to be an effective agent for the treatment of DJD.[110,111] Harmand has evaluated the effects of SAMe on human articular chondrocyte differentiation in vitro. This study demonstrated that SAMe appears to enhance native proteoglycan synthesis and secretion in human chondrocyte cultures arising from the cartilage of patients with osteoarthritis. These results point to a direct action on the cell metabolism but little is known concerning the mechanism involved.[112]

Gutierrez evaluated the effect of SAMe as a protective agent against the modifications induced by tumor necrosis factor alpha (TNF alpha) on synovial cell proliferation and extracellular matrix protein synthesis, which are hallmarks of progressive joint disease. In this study the addition of SAMe to synoviocytes incubated with TNF alpha reversed the effects induced by the cytokine. These results indicate that in cultured synovial cells, SAMe restores basal conditions after cell damage elicited by TNF alpha stimulation.[113]

Research has shown SAMe to exhibit analgesic and anti-inflammatory effects possibly by its cycloxygenase-inhibiting activity while showing no side effects involving the gastrointestinal mucosa.[110,114-116] It was shown to be similar in efficacy to several nonsteroidals.[110,114-116] Glorioso performed a double-blind multicenter study evaluating the activity of SAMe in hip and knee OA. This study was carried out to verify the effectiveness and tolerance of SAMe versus ibuprofen in 150 patients with OA. Both drugs were given twice a day for 30 days. In this study, SAMe exhibited a slightly more marked activity than the reference drug in the management of the various painful

manifestations of the joint disease. Minor side effects developed in 5 patients of SAMe group, and in 16 patients of ibuprofen group.[110]

The anti-inflammatory mechanism of SAMe was evaluated in a study by Matthews. In this study, it was found that SAMe possessed anti-inflammatory activity against carraeenen-induced inflammation in the rat. SAMe and its metabolites were found to inhibit the production of active oxygen species from rat polymorphonuclear leukocytes. SAMe also inhibited phagocytosis by stimulated cells. It did not inhbit eicosanoid synthesis in vivo and prostaglandin E2 synthesis in vitro. Results of this study suggest that the anti-inflammatory action of SAMe is in part due to its metabolites with methylthioadenosine the most significant anti-inflammatory metabolites.[117]

McNamara has described oral, disease-modifying OA agents as safe, able to cross the gastrointestinal tract, and modify the painful clinical signs associated with human and animal OA.[60,91,95] Unfortunately, the majority of reports regarding these agents for the treatment of OA are still anecdotal. In order to substantiate the efficacy of oral, disease-modifying agents, more clinical trials (random, double-blind, placebo controlled studies) are greatly needed.

RESEARCH MODELS

In order to accurately study, and successfully treat, joint injury and subsequent osteoarthritis (OA), we must first create a valid model. McIlwraith has stated the overall usefulness of an experimental model of DJD depends on its ability to mimic the naturally occurring clinical disease.[118] It is important when creating a model for the study of OA that it not be too severe. Osteoarthritis is not a single disease entity and therefore can not be treated as such. It is a heterogenous group of disorders that differ in etiology, clinical manifestations, distribution and morphology. According to Sokoloff, OA is not so much the result of the inability of articular cartilage to repair as aberration of the repair. Primary and secondary varieties reflect the "bio-" and "-mechanical" interactions in this biochemical condition and must be taken into account when creating an experimental model of OA.[119]

Creation of joint injury models has involved abrasive, chemical, and mechanical techniques.[119] Commonly employed mechanical models include transection of the cranial cruciate ligament in dogs (Pond-Nuki Model),[120,121,122] partial meniscetomy in rabbits,[119,123] osteochondral fragmentation,[124] and limb immobilization.[125]

Chemical models employ intraarticular injection of cartilage-damaging agents including Chymopapain,[55,126,127,136] sodium iodoacetate,[119,128,129] Neutral Metalloproteoglycan-

degrading enzyme (NMPE),[120] Lipopolysaccharide (LPS),[130] Chondroitinase ABC,[131] Purified collagenase,[129] and Polyene antibiotic filipin.[118]

Chymopapain (CP) is a cystine proteinase isolated from the latex of the fruits of *Carica papaya*.[132] Clinically chymopapin has been utilized for chemonnucleolysis of soft cervical dics herniations in humans. It has shown to be a minimally invasive alternative to conventional surgical disc excision.[133] However, while this is an effective mode of therapy, data clearly indicates that an optimal dose in humans still needs to be established.[134] The mechanism of action underlying chymopapain remains obscure.[135]

Williams has used polarized light (POL) to monitor changes in the organization of the articular cartilage collagen network and matrix proteoglycans (PGs) after intra-articular injection of chymopapain (CP). POL viewing of sirius red stained sections revealed a loss of normal birefringence suggesting an apparent collapse of the collagen network following intra-articular CP. Knees injected with 2.0mg CP showed no return of normal birefringence after 21 days, however, normal birefringence was found in knees injected with 0.2mg CP. Toluidine blue stained sections in the 0.2mg CP injected knees revealed a severe loss of matrix PGs followed by PG restoration. This shows that articular cartilage can recover from enzyme-induced alterations in the spatial collapse of its fibrillar network. This information refutes the fact that damage to the collagen network leads to progressive articular cartilage destruction.[127]

Metcalf performed a study evaluating the early scintigraphic appearance of papain-injected carpal joints by combined blood pool and bone phase radionuclide imaging. In this study 8 dogs had their antebrachiocarpal joint injected with 1% papain. Scintigraphy of the affected and normal carpi were then evaluated over a 28 day period. Results showed that the qualitative and quantitative scintigraphic appearances in injected carpal joints were very similar in both blood pool and bone phases. This study proves very useful as a model for studying the effectiveness of therapeutics in inflammatory joint disease.[136]

Todhunter used CP to injected into the radiocarpal joint of ponies to induce cartilage catabolism. Sequential and concurrent plasma and synovial fluid concentrations of Keratan Sulfate (KS) were then measured up to 13 month post injections. It was concluded that KS concentrations in plasma and synovial fluid may be increased in acute, marked, generalized articular cartilage catabolism.[137]

Williams demonstrated that after destruction of articular cartilage by a single injection of chymopapain into one knee of a rabbit, that the serum keratan sulfate (KS) concentration rose sharply to levels 3 to 5 times higher than the base line value. KS returned to baseline levels 7 days post chymopapain injection.[126] The increase in serum KS levels reflects changes in the metabolic activity of chondrocytes (increased catabolism of proteoglycans) in the articular cartilage.[122]

Chymopapin has been shown to exhibit anti-inflammatory properties in a study performed by Sawin. CP had the ability to modulate and reduce the activity of the preinflammatory enzyme phospholipase A2 (PLA2) and ameliorate mechanical hyperalgesia in an in vivo model of sciatica.[135]

Pelletier has demonstrated that in experimental OA, the enzymatic breakdown of the cartilage matrix by collagenolytic enzymes was modulated by synovial factors. The synovial factors then stimulate the chondrocytes to increase degradative enzyme synthesis.[120]

CP appeared to be a suitable agent for the creation of mild synovitis without cartilage damage in a study performed by Hoskinson. Histologic findings of CP injected joints reveal multifocal thickening or proliferation of synovium with slight hypervascularity. High dose CP (5 mg) produced a mild synovitis without cartilage errosion. This change was characterized histologically by difuse thickening of synovium with hypervascularity and subsynovial hypercellularity. This study did not demonstrate interlukin-1, interlukin-8, or low dose collagenase as acceptable agents for the induction of synovitis.[55]

Historically studies of OA have relied on histologic evaluation of the joints to assess the degree of injury, necessitating the death of the experimental animal and allowing only one moment in the development of disease, or response to therapy, to be

assessed. Noninvasive methods of assessment have relied on radiographs which are limited to detecting bony changes which occur relatively late in the course of DJD or identifying (but not differentiating the cause of) soft tissue swellings.

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II. SCINTIGRAPHIC EVALUATION OF GLUCOSAMINE HYDROCHLORIDE AND CHONDROITIN SULFATE AS TREATMENTS FOR ACUTE SYNOVITIS IN DOGS

The following portion of this thesis contains a paper, which was accepted for publication to the American Journal of Veterinary Research on February 28, 1999. In addition, a portion of this work was presented at the 8th Annual American College of Veterinary Surgeons Symposium, Small Animal Orthopedic Surgery Scientific Session, Chicago, IL. October 9,1998.

Scintigraphic evaluation of glucosamine hydrochloride and chondroitin sulfate as treatments for acute synovitis in dogs

Sherman O. Canapp Jr, DVM, MS; Ronald M. McLaughlin Jr, DVM, DVSc; James J. Hoskinson, DVM; James K. Roush, DVM, MS; Mike D. Butine, PhD

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From the Department of Clinical Science, College of Veterinary Medicine, (Canapp, McLaughlin, Hoskinson, Roush), and the Department of Statistics (Butine), Kansas State University, Manhattan, KS 66506. Dr. Canapp's present address is College of Veterinary Medicine, Department of Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO 65211. Dr. McLaughlin's present address is Indianapolis Veterinary Specialists, 8250 Bash St., Indianapolis, IN 46250. Dr. Butine's present address is Immunex Corporation, 51 University Street, Seattle, WA 98101.

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Objective—To evaluate the effects of orally administered glucosamine hydrochloride (GlAm)–chondroitin sulfate (CS) and GlAm–CS–S-adenosyl-L-methionine (SAMe) on chemically induced synovitis in the radiocarpal joint of dogs.

Animals—32 adult mixed-breed dogs.

Procedure—For 21 days, all dogs received a sham capsule (3 groups) or GlAm-CS (prior treatment group) in a double-blinded study. Unilateral carpal synovitis then was induced by injecting the right radiocarpal joint with chymopapain, and the left radiocarpal joint (control joint) with saline (0.9% NaCl) solution. Joints were injected on alternate days for 3 injections. After induction of synovitis, 2 groups receiving sham treatment were given GlAm-CS or GlAm-CS–SAMe. The fourth group continued to receive sham capsules (control group). Joint inflammation was quantified, using nuclear scintigraphy (before injection of joints and days 13, 20, 27, 34, 41, and 48 after injection). Lameness evaluations were performed daily.

Results—Dogs given GlAm-CS before induction of synovitis had significantly less scintigraphic activity in the soft-tissue phase 48 days after joint injection, had significantly less uptake in the bone phase 41 and 48 days after joint injection, and had significantly lower lameness scores on days 12 to 19, 23, and 24 after injection, compared with other groups.

Conclusions and Clinical Relevance—Analysis of results of this study suggest that prior treatment with GlAm-CS for 21 days had a protective effect against chemically induced synovitis and associated bone remodeling. Prior treatment with GlAm-CS also reduced lameness in dogs with induced synovitis.

Joint injury and osteoarthritis (OA) are common in animals.[1] Osteoarthritis has been described as one of the most frequently encountered joint diseases in dogs.[2]

Although the cause of OA varies, initial changes in many animals include inflammation of the synovial membranes and joint capsule without cartilage damage. The inflamed synovial membrane (synovitis) allows leukocytes to invade the joint space. Synovial cells and leukocytes release destructive mediators (free radicals, cytokines, and prostaglandins) into the joint cavity, which further inflame the synovium and modulate chondrocyte metabolism, damaging the articular cartilage matrix.[3] Synovitis is responsible for a majority of the pain in osteoarthritic joints.[4]

Treatment for animals with joint disease has limited success once bony changes associated with degeneration have developed. Preventing or minimizing the inflammatory process within the synovium may interrupt the cascade of events that can lead to cartilage degeneration. Controlling or preventing synovitis early in the disease process may slow the progression of degenerative joint disease (DJD). Currently, the ideal treatment for synovitis has not been found, although the identification of such treatment is essential if the disease is to be controlled before irreversible osteoarthritis develops.[5]

Traditionally, corticosteroids and nonsteroidal anti-inflammatory drugs (NSAID) have been used for the treatment of OA. However, long-term use of these agents may produce undesirable adverse effects and also can have deleterious effects on chondrocyte

and matrix homeostasis.[6] The apparent impact of some NSAID on the progression of DJD may be attributable to the inhibitory activity on the synthesis of prostaglandins.[7] Unfortunately, many NSAID contribute to generation of catabolic cytokines, cause chondrocytes to degrade matrix, or prevent expression of anabolic cytokines, which results in progression of OA.[8]

Attention recently has shifted to alternative methods of treatment for medical management of OA that focus on slowing the process of cartilage degradation and promoting synthesis of cartilage matrix.[9] Research has led to the marketing of orally administered disease-modifying OA agents that may directly influence chondrocyte metabolism in a beneficial manner.[8] These disease-modifying OA agents have been defined as compounds that support or enhance macromolecular synthesis by chondrocytes (DNA, RNA, collagen, proteoglycans, matrix), support or enhance synthesis of hyaluronan by synoviocytes, inhibit degradative enzymes (eg, collagenase, cathepsins, hyaluronidase, chondroitonases) or inflammatory mediators (interleukin–1, tumor necrosis factor, prostaglandin E_2), and remove or prevent formation of fibrin, thrombi, and plaques in synovia or subchondral blood vessels.[10] Orally administered disease-modifying OA agents reportedly counter the destructive inflammatory process and encourage normalization of synovial fluid and cartilage matrix.[11,12] Compounds capable of these actions are identical to compounds used by the body to manufacture proteoglycans, specifically glycosaminoglycans (GAG).[13,14]

Glucosamine hydrochloride (GlAm) and chondroitin sulfate (CS) in purified, extracted, or complexed form are the main compounds in most orally administered disease-modifying OA agents. Glucosamine hydrochloride is an amino-monosaccharide nutrient and precursor of the disaccharide unit of GAG, which is the building block of the ground substance of articular cartilage (proteoglycans).[11] Chondroitin sulfate is a long-chain polymer of a repeating disaccharide unit (galactosamine sulfate and glucuronic acid).[15] It is the predominant GAG found in articular cartilage and is a natural component of several other body tissues (tendons, bones, vertebral discs, and cardiac and corneal tissues).[15]

S-adenosyl-L-methionine (SAMe) is a physiologic molecule that is found in living cells and participates in a number of intermediate metabolic reactions.[16] Clinical studies have documented that SAMe can be an effective agent for treatment of DJD.[17,18] S-adenosyl-L-methionine has analgesic and anti-inflammatory effects, possibly as a result of its cycloxygenase-inhibiting activity, and it is similar in efficacy to several NSAID.[17,19-20] The purpose of the study reported here was to evaluate the effects of orally administered GlAm-CS and GlAm-CS-SAMe on chemically induced synovitis in the radiocarpal joint of dogs.

Materials and Methods

Dogs—Thirty-two skeletally mature mixed-breed dogs that were between 1 and 5 years old and weighed between 4.5 and 11 kg were used in the study. None of the dogs had

clinical or radiographic evidence of disease of the carpal joints, and results of hematologic analysis were typical of clinically normal dogs.

A minimum of 7 days was allowed to acclimate dogs prior to initiation of the study. Initial physical, radiographic, and nuclear scintigraphic examinations were performed on all dogs during this period.

Complete physical examinations (heart rate, respiratory rate, mucous membrane color, capillary refill time, appetite, water consumption, and musculoskeletal soundness) were conducted daily throughout the acclimation period and subsequent study. Data were recorded daily in each dogs record. Rectal temperature was recorded once weekly, except when any of the aforementioned variables appeared abnormal.

Compounds—Each capsule of the agents[a] used in the study contained a combination of purified FCHG49 ™ glucosamine hydrochloride (500 mg) [b], TRH122 ™ sodium chondroitin sulfate (400 mg) [c], manganese (10 mg), and ascorbate (66 mg).[5] The second agent used in the study consisted of these same ingredients but also contained SAMe (200 mg).[d] The dose of GIAm–CS used in this study was higher than the manufacture's recommended dose for treatment of DJD.

Procedures—Dogs were randomly allocated to 4 groups (8 dogs/group), using the Moses-Oakford treatment assignment worksheet.[21] Prior to induction of synovitis, all

dogs were orally administered sham treatment (3 groups) or GIAm-CS (prior treatment group) at 8-hour intervals for 21 days.

After this 3-week period, each dog was given an IM injection of butorphanol tartrate[e] (0.05 mg/kg) and acepromazine maleate[f] (0.05 mg/kg). The skin over each radiocarpal joint was clipped and prepared in a sterile manner. Unilateral carpal synovitis was induced by injecting the right radiocarpal joint with 0.2 ml (20 U) of chymopapain.[g] The left radiocarpal joint (control joint) was injected with 0.2 ml of saline (0.9% NaCl) solution. Joints were injected on alternate days for 3 injections (day 1, 3, 5). All dogs received butorphanol (0.05 mg/kg, IM) as needed for analgesia during the induction period.

After induction of synovitis (day 6), 2 groups were treated with GIAm-CS and GIAm-CS-SAMe, respectively. Dogs in the prior treatment group continued to receive the same dose of GIAm-CS, whereas the fourth group continued to receive sham treatment. All investigators were unaware of the group to which each dog was assigned, and all medications were unmarked and packaged in containers labeled only with the group number.

Two-phase bone scintigraphy was performed on all dogs prior to joint injection and on days 13, 20, 27, 34, 41, and 48 after induction of synovitis. At each evaluation, dogs received 37 Mbq of technetium Tc 99m methylenediphosphonate/kg of body

weight, which was administered via a saphenous vein. Palmar and lateral images of both carpal joints were obtained 2 minutes (soft-tissue phase) and 2 hours (bone phase) after injection.

Images (30 seconds) were acquired in a 150 X 150 X 16 bit matrix, using a large field-of-view gamma camera equipped with a general purpose collimator.[h] The camera was photo peaked at 140 KeV with a 15% window. Images were acquired on a dedicated nuclear medicine computer [i] and stored on a removable hard drive.

An identically sized region of interest was manually drawn for each carpus and mid antebrachium scintigraph (Fig 1). Ratio of activity for the synovitis vs control limb was calculated, using the following formula:

$$\text{synovitis index} = \frac{\text{synovitis carpus/synovitis antebrachium}}{\text{control carpus/control antebrachium}} \times 100$$

Synovitis index represented increased activity in the affected limb expressed as a percentage of the control limb. Nuclear scintigraphs, region of interest for each scintograph, and final ratios were evaluated by a radiologist who was unaware of treatment given to each dog.

Lameness evaluation was initiated on day 6 (ie, the day after the final chymopapain injection) and continued daily until end of the study. Evaluations were performed at the same time each day by investigators (SC, RM) who were unaware of the

group to which each dog was assigned. Lameness was scored by marking a 10-cm line scale (visual analog scale) according to the lameness of each dog (sound = 0 cm, unable to bear weight = 10 cm).

Statistical analysis—Lameness scores and scintigraphic data were analyzed by use of a repeated-measures ANOVA. Mixed-models software [j] accommodated heterogeneous variances as well as temporal covariances. Pairwise comparisons were made, using *t*-tests, with appropriately estimated SEM and adjusted degrees of freedom. Significance was defined as $P \leq 0.05$.

Results

Lameness evaluation—All dogs developed clinical evidence of synovitis (carpal swelling, lameness) without signs of systemic illness. Complications associated with administration of sham treatment, GIAm-CS, or GIAm-CS-SAMe were not detected during the study. All dogs were completely unable to bear weight (lameness score of 10) after the third injection of chymopapain (day 5). None of the dogs required administration of analgesics after day 5.

Dogs given prior treatment with GIAm-CS for 21 days had a significant decrease in lameness on days 12 to 19, 23, and 24, compared with other groups (Fig 2). All dogs were sound (lameness score of 0) by day 32 (27 days after the final chymopapain injection). Significant differences were not detected at any time among the 3 groups that did were not given GIAm-CS prior to induction of synovitis (data not shown).

Scintigraphy—An initial increase was evident in the ratio of activity for soft-tissue and bone phases in chymopapain-injected joints, which was followed by a slow decrease until day 48. Dogs given GIAm-CS for 21 days before induction of synovitis had significantly ($P = 0.01$) less activity during the soft-tissue phase on day 48, compared with other groups (Fig 3). Dogs given GIAm-CS for 21 days prior to induction synovitis had significantly less uptake during the bone phase on day 41 ($P = 0.02$) and 48 ($P = 0.04$), compared with other groups (Fig 4). There was not a significant difference at any time period among the groups that were not given GIAm-CS prior to induction of synovitis (data not shown). Representative soft-tissue (Figures 5a and 5b) and bone (Figures 6a and 6b) scintigraphs from dogs receiving treatment prior to induction of synovitis and dogs given sham treatment were obtained prior to treatment and on days 20 and 48.

Discussion

In the study reported here, chymopapain injected into the left radiocarpal joints induced clinical and scintigraphic evidence of transient synovitis. This was consistent with previous findings in which intraarticular injections of chymopapain caused substantial synovitis and variable amounts of articular cartilage damage.[k,22-26] Histologic findings for radiocarpal joints of dogs similarly injected with chymopapain revealed multifocal thickening or proliferation of synovium with slight hypervascularity and subsynovial hypercellularity.[k]

In the past, studies of OA relied on histologic evaluation of joints to assess efficacy of medical treatment. This required that animals be euthanatized and allowed assessment of only a single moment in progression of the disease. Noninvasive methods of assessment have relied on radiographs, which are limited to detecting bony changes

that become apparent relatively late in the course of DJD, or techniques for identifying (but not differentiating the cause of) soft-tissue swellings. Nuclear scintigraphy was used in this study, because it can noninvasively detect and quantify some changes that are evident during an episode of synovitis in a joint and allows the joint to be serially evaluated in a living animal; therefore, response to treatment is readily assessed over time. The 2 to 5-minute period immediately after injection of the radioisotope is termed the soft-tissue phase. Images obtained during the soft-tissue phase provide a relatively sensitive but nonspecific method for evaluation of joint inflammation. During this period, the radioactive material is distributed throughout the extracellular fluid. Although some uptake by bone may be evident during the first 5 minutes after injection, bone activity, unless extreme, is obscured by activity in the extracellular fluid. For this reason, bone-phase images are obtained 2 to 3 hours after injection of the radioisotope, when extracellular fluid activity has been cleared. In other studies,[k,27-30] it was documented that nuclear scintigraphy is an effective method of quantifying synovial inflammation.

The significant decrease in ratios of activity evident in the soft-tissue (day 48) and bone (days 41 and 48) phases in dogs given GIAm–CS prior to induction of synovitis suggest that preventive treatment may have a protective effect for synovitis. Although the ratios of activity in the soft-tissue and bone phases continued to decrease in this group until the end of the study, values for the groups that did not receive treatment prior to induction of synovitis appeared to plateau.

Analysis of results of subjective lameness evaluations also supported the fact that treatment with GIAm–CS before induction of synovitis had a positive effect. Dogs that received treatment with GIAm-CS before induction of synovitis had a significantly lower lameness score 1 to 3 weeks (days 12 to 19, 23, 24) after induction of synovitis. The 10-

cm line scale was used, which enabled us to perform a linear method of statistical analysis with continuous rather than discrete data.

In the study reported here, efficacy of GIAm-CS as a treatment for synovitis was not compared with that for other known anti-inflammatory agents. The objective of the study was to evaluate the agents in dogs, using a chemical method for inducing synovitis, and determine whether there were any anti-inflammatory effects. The impetus for the study was clinical experiences in which lameness and joint inflammation in some dogs subjectively improved relatively quickly after administration of GIAm-CS, an improvement that was evident more quickly than could be produced by gradual incorporation of the compound into articular cartilage. On the basis of these clinical experiences, it was believed that some of the reported benefits of the compounds for use in controlling lameness, and eventually for use in improving joint health, were perhaps a result of a suppression of the inflammatory cascade within a diseased joint. Analysis of results of the study reported here indicated that the compounds do decrease synovitis, providing treatment was initiated before joint inflammation was induced. Comparison with known anti-inflammatory medications is warranted to determine the relative efficacy of GIAm-CS in reducing joint inflammation.

During the course of our study, differences were not seen between the sham-treatment group and groups given GIAm-CS or GIAm-CS-SAMe after induction of synovitis. Differences were only identified between the group given GIAm-CS before induction of synovitis and the sham-treatment group, suggesting that GIAm-CS at the dose administered had a protective effect for acute synovitis. The short duration of the study and severe synovitis created may have prevented identification of a therapeutic effect of GIAm-CS and GIAm-CS-SAMe; future studies should be of longer duration to

address this concern. Persistence of decreased activity for soft-tissue and bone phases in the group given GIAm-CS before induction of synovitis raises the possibility that the protective effect for acute synovitis may extend into long-term effects (ie, protective against OA). Long- term studies and studies that use clinically affected dogs after naturally developing joint injury would be helpful in evaluating this possibility.

None of the dogs had adverse reactions to the administered medications. This concurs with studies that documented the safety of these agents in dogs.[31]

Although the scope of this study did not investigate the mechanism of action for GIAm and CS, several explanations have been offered for the protective effects discovered. Biochemical and pharmacologic studies revealed that administration of GIAm tends to normalize cartilage metabolism and stimulate synthesis of proteoglycans so that articular function is partially restored.[32] Glucosamine hydrochloride stimulates synovial fibroblasts to synthesize hyaluronic acid.[32] Furthermore, GIAm is used to stimulate synthesis of hyaluronic acid by synoviocytes and of all other GAG after conversion to amino sugars by epimerases. It has been suggested that GIAm is more than just a building block for GAG; it also increases cartilage matrix synthesis. Glucosamine hydrochloride stimulates GAG, proteoglycan, and collagen synthesis in chondrocytes and fibroblasts.[12, 13] Chondroitin sulfate, through its long-chain length, competitively inhibits enzymes that degrade proteoglycans in cartilage and synovium.[13,34] Chondroitin sulfate substantially increases intrinsic viscosity and the concentration and hydrodynamic size of hyaluronate.[1] These agents work synergistically to form GAG, inhibit degradative enzymes, and increase cartilage metabolism and matrix production.[14,37,38] The ability of GIAm-CS to inhibit lysosomal enzymes and prevent glycosaminoglycan and collagen

breakdown may have contributed to the decreased synovial inflammation and radionucleotide uptake evident in the dogs of our study.

The efficacy of orally administered disease-modifying OA agents in the treatment of OA remains controversial. A synergistic effect has been documented from the combined use of GIAm and CS for the treatment of OA.[13,32-33,35-37,41,m,n] Our findings in the group given GIAm-CS before induction of synovitis suggest that continuous administration of GIAm-CS to dogs with DJD may be beneficial by decreasing the inflammatory response to acute exacerbations of the disease or, possibly, by reducing long-term bone responses. Marketed orally administered disease-modifying OA agents are widely used and vary in their source, composition, and purity of compounds.[39-41] Currently, there are not any United States Pharmacopeia standards established for orally administered disease-modifying OA agents.[39] Our results should be considered specific for the agents used in our study and should not be extrapolated to apparently similar products. Additionally, the study reported here only evaluated the effect of the compounds on joint inflammation. Other important aspects of DJD, including changes in articular cartilage and subchondral bone, were not specifically examined. Additional studies are required to fully elucidate the effects of GIAm-CS on all aspects of DJD.

Footnotes

[a]Cosequin DS, Nutramax Laboratories, Edgewood, Md.

[b]FCHG49[tm]glucosamine hydrochloride, Nutramax Laboratories, Edgewood, Md.

[c]TRH122[tm]sodium chondroitin sulfate, Nutramax Laboratories, Edgewood, Md.

[d]SAMe, Knoll Pharmaceutical, Milano, Italy.

[e]Butorphanol tartrate, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.

[f]Acepromazine maleate, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.

[g]Chymopapain, Sigma Chemical Co, St. Louis, Mo.

[h]750 ZLC Siemens, Isleton, NJ.

[i]Nuclear Mac, Scientific Imaging, Littleton, Colo.

[j]SAS Institute Inc, Cary, NC.

[k] Hoskinson JJ, Gaughan EM, McLaughlin RM, et al. Scintigraphic evaluation of synovitis. (abstr). In, *Proceedings*. 20th Annual Meeting of the Veterinary Orthopedic Society, Lake Louise, Canada. Feb. 1993. p 44.

[l]Ronca G. Anti-inflammatory activity of chondroitin sulfate. In, *Proceedings*. 3rd Int Congr Osteoarthritis Res Soc Osteoarthritis in Focus: etiopathogenesis, assessment, and treatment (abstr).1997;5 (Suppl A):69.

[m]McNamara PS, Barr SC, Idouraine A, et al. Effects of an oral chondroprotective agent (Cosequin) on cartilage metabolism and canine serum (abstr). In, *Proceedings*. 24th Annu Conf Vet Orthoped Soc 1997;p35.

"Hulse DS, Hart D, Slatter M, et al. The effect of Cosequin in cranial cruciate deficient and reconstructed stifle joints in dogs (abstr). In, *Proceedings*. 25th Annu Conf Vet Orthoped Soc 1998;p64.

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Figure legends

Figure 1. Palmar scintographic view of the forelimbs of a dog obtained 2 minutes after injection of radioisotope. Region of interest is indicated for the carpal joints (squares) and the antebrachium (circles).

Figure 2. Comparison of lameness scores for 1 group of dogs given glucosamine hydrochloride (GIAm) and chondroitin sulfate (CS) prior to induction of synovitis (injections administered on days 1, 3, and 5) and 3 groups of dogs that were not given GIAm-CS prior to induction of synovitis. Dogs given treatment prior to induction of synovitis had a more rapid improvement in lameness score. Values represent least-squares means. *Values differed significantly ($P \leq 0.05$) between groups.

Figure 3. Comparison of least-square mean values for the synovitis index (soft-tissue phase) of dogs given GIAm-CS prior to induction of synovitis and dogs not given GIAm-CS prior to induction of synovitis. *Values were significantly different ($P \leq 0.05$) between groups.

Figure 4. Comparison of least-squares mean values for the synovitis index (bone phase) of dogs given GIAm-CS prior to induction of synovitis and dogs not given GIAm-CS prior to induction of synovitis. *Values were significantly lower ($P \leq 0.05$) between groups.

Figure 5a. Palmar scintigraphic views obtained during the soft-tissue phase for dogs representative of the group given GIAm-CS prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

Figure 5b. Palmar scintigraphic views obtained during the soft-tissue phase for dogs representative of the group given sham treatments prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

Figure 6a. Palmar scintigraphic views obtained during the bone phase for dogs representative of the group given GIAm-CS prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

Figure 6b. Palmar scintigraphic views obtained during the bone phase for dogs representative of the group given sham treatments prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

III. APPENDICES

Region of Interest

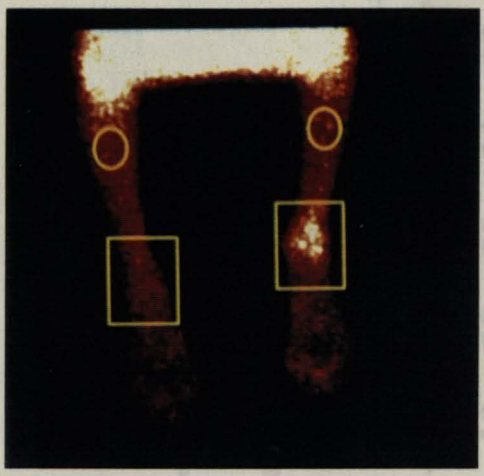

Figure 1. Palmar scintographic view of the forelimbs of a dog obtained 2 minutes after injection of radioisotope. Region of interest is indicated for the carpal joints (squares) and the antebrachium (circles).

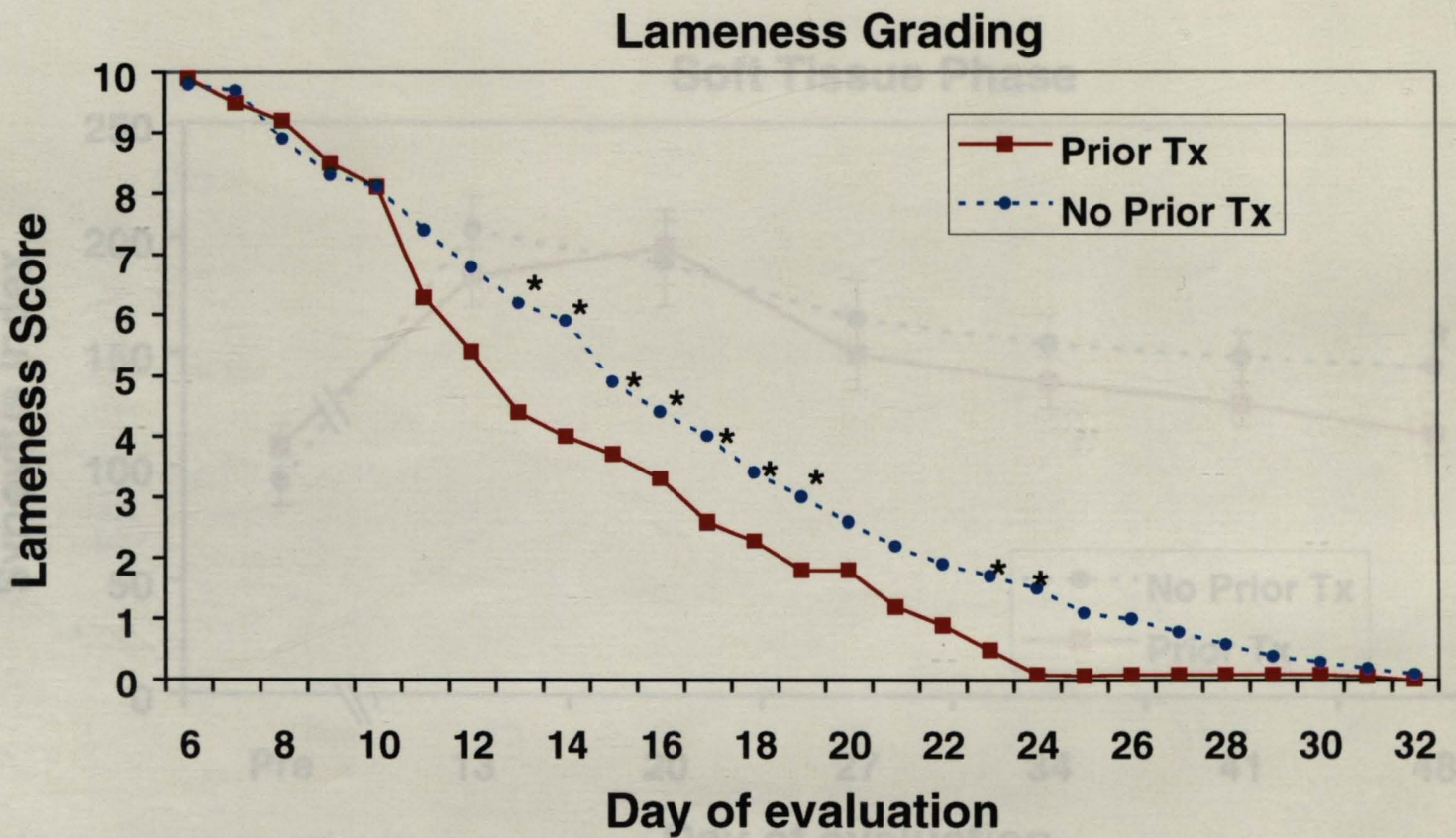

Figure 2. Comparison of the lameness scores for dogs given GIAm-CS prior to induction of synovitis (Prior Tx) and dogs that were not given GIAm-CS prior to induction of synovitis (No Prior Tx). Values represent least-squares means. *Values differed significantly ($P \leq 0.05$) between groups.

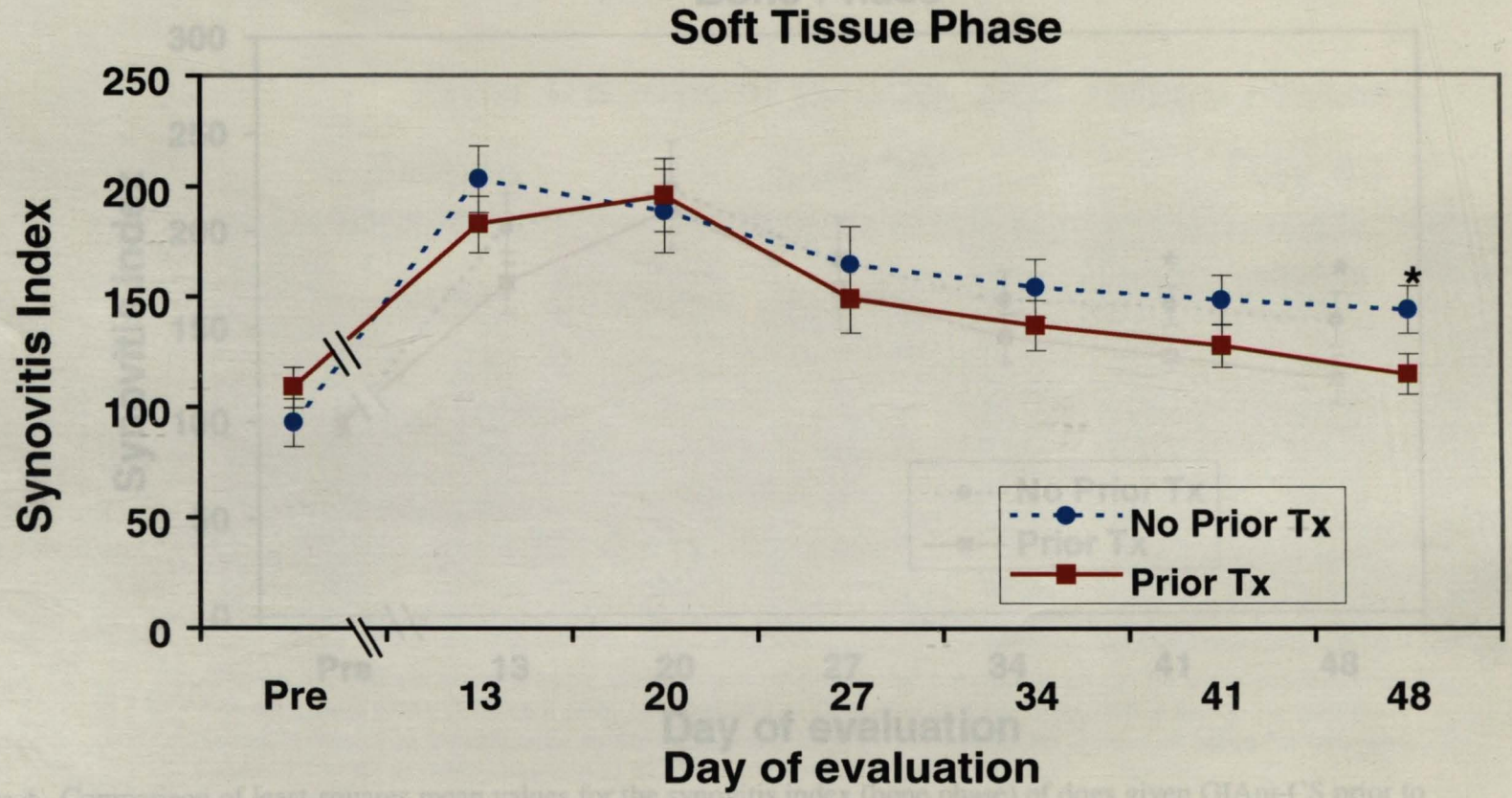

Figure 3. Comparison of least-square mean values for the synovitis index (soft-tissue phase) of dogs given GIAm-CS prior to induction of synovitis (Prior Tx) and dogs not given GIAm-CS prior to induction of synovitis (No Prior Tx). *Values were significantly different ($P \leq 0.05$) between groups.

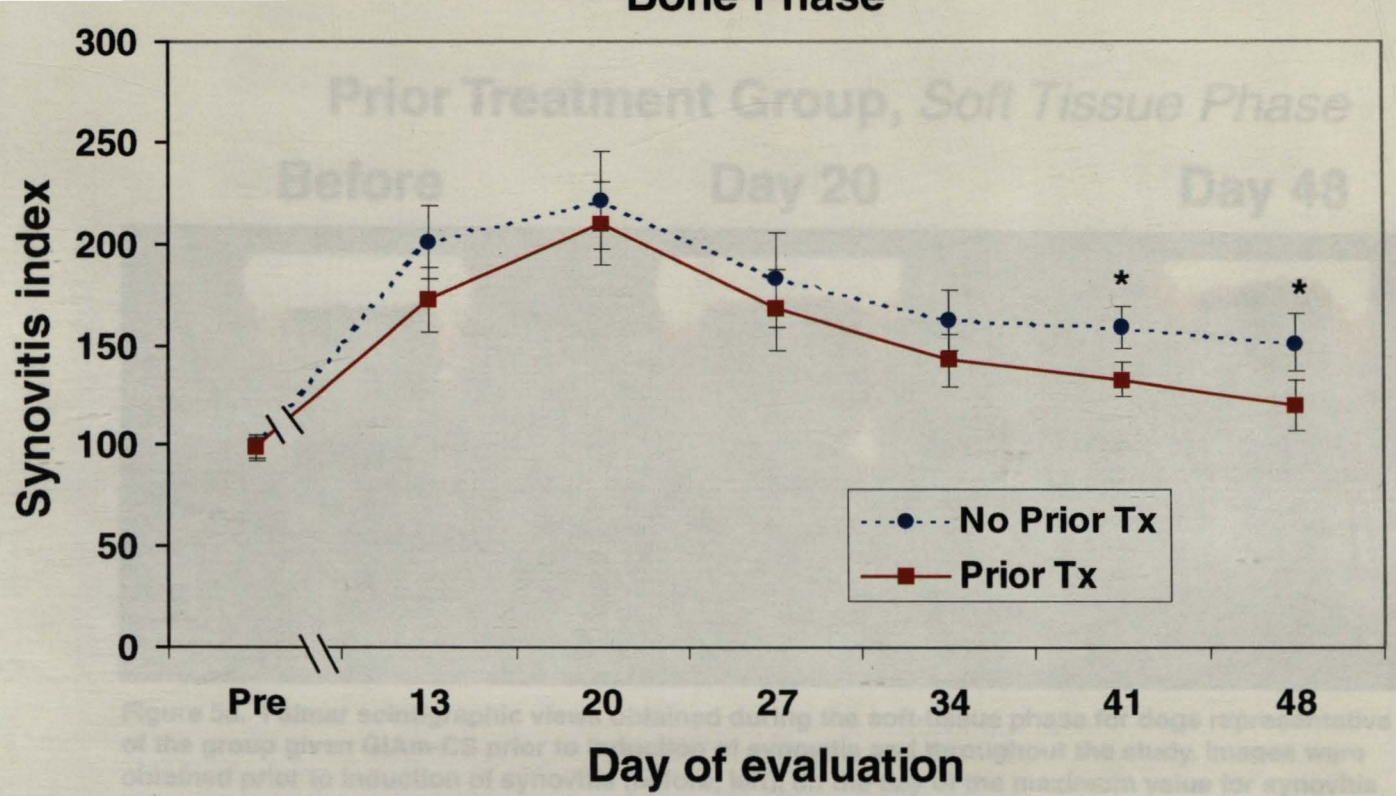

Figure 4. Comparison of least-squares mean values for the synovitis index (bone phase) of dogs given GIAm-CS prior to induction of synovitis (Prior Tx) and dogs not given GIAm-CS prior to induction of synovitis (No Prior Tx). *Values were significantly lower ($P \leq 0.05$) between groups.

Prior Treatment Group, *Soft Tissue Phase*

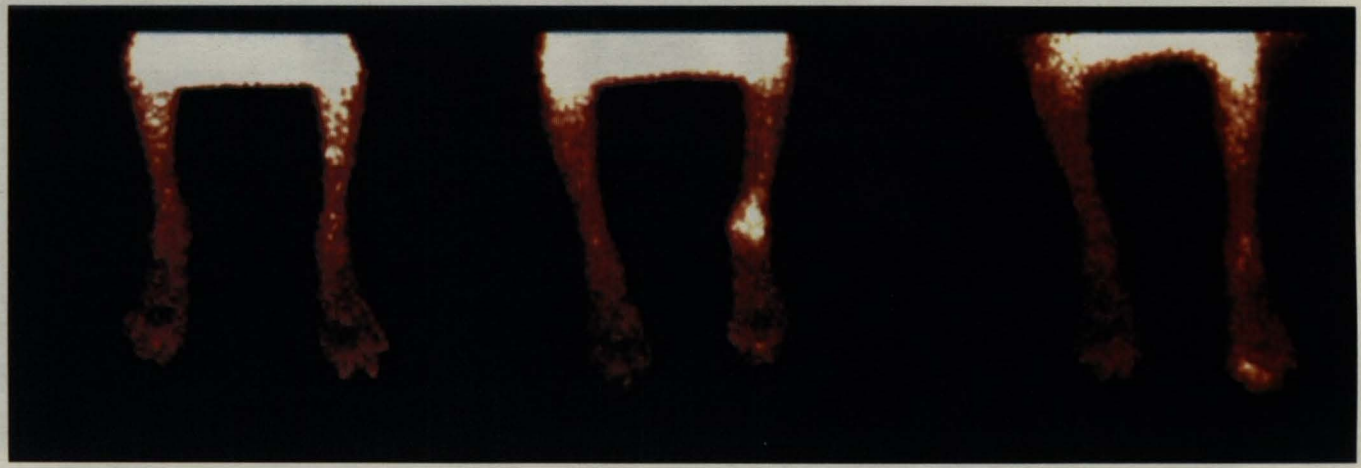

Figure 5a. Palmar scintigraphic views obtained during the soft-tissue phase for dogs representative of the group given GlAm-CS prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

Sham Treatment Group, *Soft Tissue Phase*

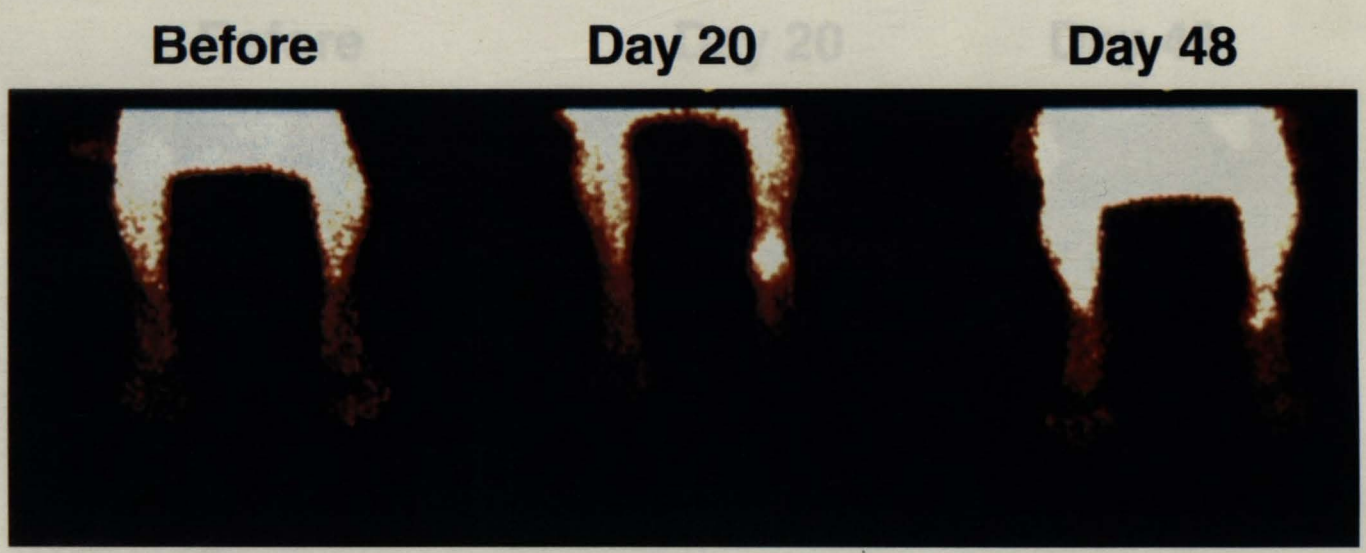

Figure 5b. Palmar scintigraphic views obtained during the soft-tissue phase for dogs representative of the group given sham treatments prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

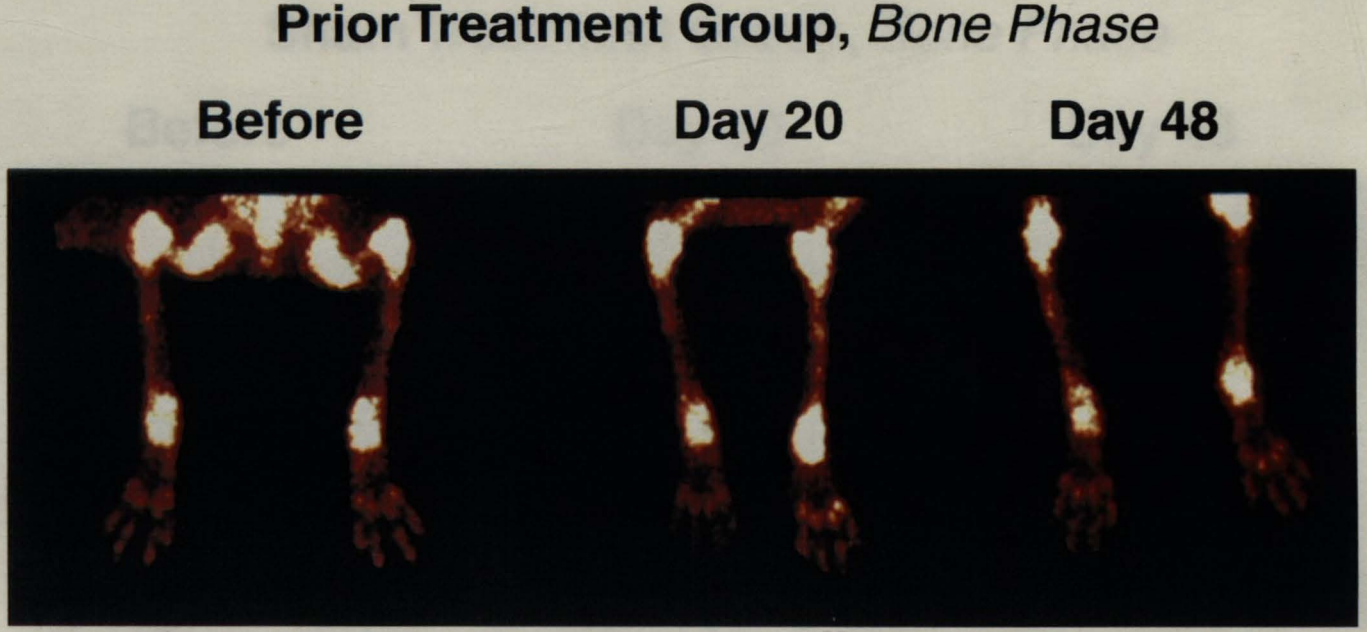

Figure 6a. Palmar scintigraphic views obtained during the bone phase for dogs representative of the group given GlAm-CS prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).

Sham Treatment Group, *Bone Phase*

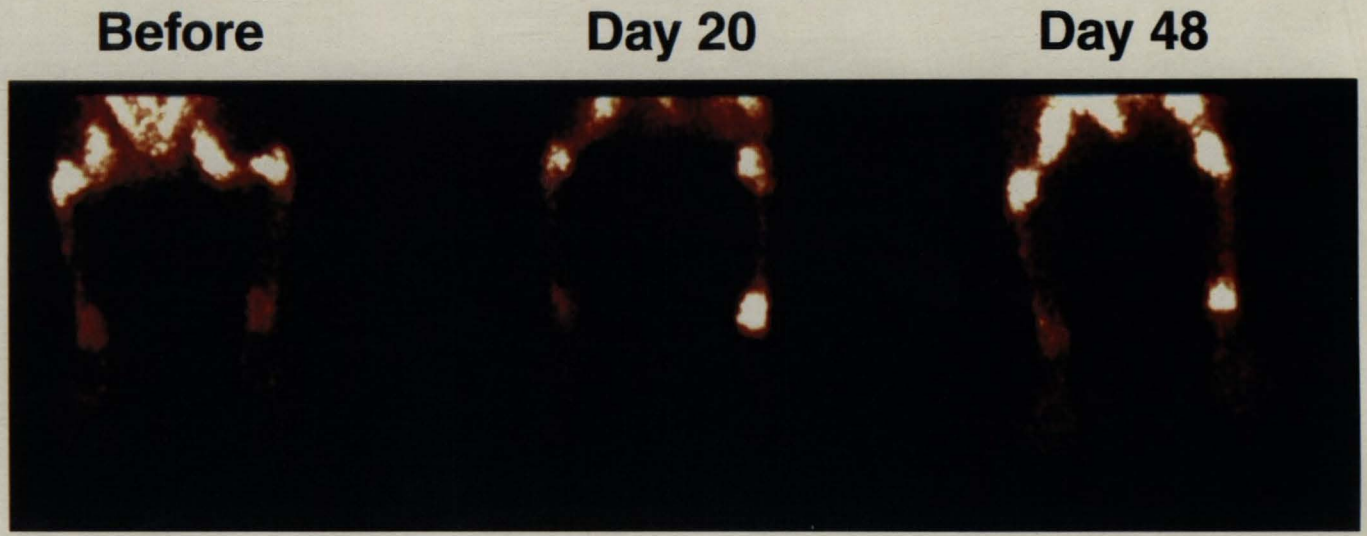

Figure 6b. Palmar scintigraphic views obtained during the bone phase for dogs representative of the group given sham treatments prior to induction of synovitis and throughout the study. Images were obtained prior to induction of synovitis (before; left), on the day of the maximum value for synovitis index (day 20; middle), and at end of the study (day 48; right).