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The Pathobiology of Osteoarthritis and the Rationale for the Use of Pentosan Polysulfate for Its Treatment

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Objectives: Structure-modifying osteoarthritis (OA) drugs (SMOADs) may be defined as agents that reverse, retard, or stabilize the underlying pathology of OA, thereby providing symptomatic relief in the long-term. The objective of this review was to evaluate the literature on sodium pentosan polysulfate (NaPPS) and calcium pentosan polysulfate (CaPPS), with respect to the pathobiology of OA to ascertain whether these agents should be classified as SMOADs.
Methods: Published studies on NaPPS and CaPPS were selected on the basis of their relevance to the known pathobiology of OA, which also was reviewed.
Results: Both NaPPS and CaPPS exhibit a wide range of pharmacological activities. Of significance was the ability of these agents to support chondrocyte anabolic activities and attenuate catabolic events responsible for loss of components of the cartilage extracellular matrix in OA joints. Although some of the anti-catabolic activities may be mediated through direct enzyme inhibition, NaPPS and CaPPS also have been shown to enter chondrocytes and bind to promoter proteins and alter gene expression of matrix metalloproteinases and possibly other mediators. In rat models of arthritis, NaPPS and CaPPS reduced joint swelling and inflammatory mediator levels in pouch fluids. Moreover, synoviocyte biosynthesis of high-molecular-weight hyaluronan, which is diminished in OA, was normalized when these cells were incubated with NaPPS and CaPPS or after intraarticular injection of NaPPS into arthritic joints. In rabbit, canine, and ovine models of OA, NaPPS and CaPPS preserved cartilage integrity, proteoglycan synthesis, and reduced matrix metalloproteinase activity. NaPPS and CaPPS stimulated the release of tissue plasminogen activator (t-PA), superoxide dismutase, and lipases from vascular endothelium while concomitantly decreasing plasma levels of the endogenous plasminogen activator inhibitor PAI-1. The net thrombolytic and lipolytic effects exhibited by NaPPS and CaPPS may serve to improve blood flow through subchondral capillaries of OA joints and improve bone cell nutrition. In geriatric OA dogs,

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NaPPS and CaPPS reduced symptoms, as well as normalized their thrombolytic status, threshold for platelet activation, and plasma triglyceride levels. These hematologic parameters were shown to be abnormal in OA animals before drug treatment. Similar outcomes were observed in OA patients when CaPPS or NaPPS were given orally or parenterally in both open and double-blind trials. ***Conclusions:*** **The data presented in this review support the contention that NaPPS and CaPPS should be classified as SMOADs. However, additional long-term clinical studies employing methods of assessing joint structural changes will be needed to confirm this view.**

INDEX WORDS: Osteoarthritis; treatment; pentosan polysulfate; structural modifying osteoarthritis drugs (SMOADs).

ALTHOUGH RARELY life-threatening, osteoarthritis (OA) and associated musculoskeletal pain and disability are the major causes of morbidity in the United States (1, 2) and other developed nations (3, 4). Radiological assessment of OA indicates a prevalence in middle-aged individuals of approximately 80%, and this increases markedly with aging, being almost universal by the seventh decade (5). The socioeconomic consequences of OA are considerable and are escalating as the longevity of the elderly population rises. It has been estimated that the cost of managing OA together with other forms of arthritis in the United States in 1996 exceeded $70 billion (6).

The causes of OA are multifactorial, and although aging is the most strongly associated risk, mechanical, hormonal, and genetic factors all contribute to varying degrees. OA emerges as a clinical syndrome when these determinants result in sufficient joint damage to cause impairment of function and the appearance of symptoms. This clinical syndrome is manifest radiologically by joint space narrowing (due to loss of cartilage) and extensive remodeling of subchondral bone with proliferation at the joint margins (osteophytosis) (7, 8). In the late stages of disease, these joints are characterized pathologically by extensive cartilage fibrillation, loss of staining for proteoglycans (PGs), and eburnation of bone at sites of high contact stress. Where cartilage is still intact, invasion of the subchondral vasculature into calcified cartilage occurs, accompanied by advancement of the tidemark (9-11). Although some chondrocytes may still proliferate and release PGs territorially, many are nonviable, as shown by loss of nuclear staining or empty lacunae (12). The subchondral bone beneath the regions denuded of cartilage is generally sclerotic and consists of immature woven bone. However, in deeper regions, the trabecular numbers of the cancellous bone may be decreased and intertrabecular space increased (13). Histological studies have shown that the marrow spaces of the cancellous bone, particularly in the hip joint, may become engorged with lipid, cholesterol, and fibrin deposits (14, 15). These lipid emboli and thrombi can impede blood flow, leading to ischemia and necrosis of bone cells (16-19).

Products of cartilage breakdown have been shown to be antigenic (20-22), and when released into synovial fluid due to excessive catabolism, may provoke synovitis. This has been shown at arthroscopic examination (23), histologically (24, 25), and immunohistologically (26) in tissues obtained at the time of joint replacement surgery. This synovial inflammation, once established, can alter the metabolism of resident synoviocytes, the major biosynthetic source of hyaluronan (HY) in synovial fluid (27, 28). Inflammatory mediators released from local synovial cells and infiltrating leukocytes can promote increased vascular permeability and the accumulation of plasma in synovial fluid, thereby decreasing HY concentration. This dilution of HY and reduction in its molecular weight due to abnormal synthesis by synoviocytes results in a decrease in the viscoelasticity of synovial fluid and thus its ability to lubricate and protect articular cartilage (29). Macrophages of the synovium, together with the leukocytes that have entered the synovial cavity, are also abundant source of cytokines (30), procoagulant factors, proteinases (31), and oxygen-derived free radicals (32), including nitric oxide (NO·) (33, 34). Although much of the

excess proteolytic activity released into synovial fluid is abrogated by the endogenous inhibitors present (35, 36), cytokines and free radicals can freely diffuse into cartilage and downregulate proteoglycan and collagen synthesis by chondrocytes. These cytokines also can initiate the production of catabolic proteinases, cytokines, and free radicals such as NO· by the cartilage cells, which contribute to further matrix destruction through paracrine pathways (37).

Failure of cartilage and bone in OA has the potential to both initiate and sustain disease progression by a variety of interrelated pathways. In Figure 1, an attempt has been made to represent these routes diagrammatically, and although this schema is an oversimplification of a complex and evolving paradigm, it serves to highlight potential sites for pharmacological intervention in OA.

Pharmacological management in OA has, up until quite recently, targeted the symptoms of the disease rather than the underlying cause; analgesics, steroidal, and nonsteroidal antiinflammatory drugs (NSAIDs) represent the mainstay of treatment (38-43). However, the deleterious side effects associated with the use of many of these agents (44-49) has led to a more conservative approach to their use in recent years.

The discovery of two isoforms of cyclooxygenase, the enzymes encoded by different genes and responsible for the conversion of arachidonic acid into prostaglandin E_2 (PGE_2), has provided fresh impetus for the use of NSAIDs in the management of the OA patient. In the gastrointestinal tract, a constitutive form of cyclooxygenase, COX-1, is mainly responsible for the synthesis of eicosanoids, including PGE_2, which in these tissues serves to inhibit acid secretion and preserve mucosal integrity (50-53). The other form of cyclooxygenase, COX-2, is not normally stored, but its synthesis is rapidly induced when the cell is stimulated by exposure to phorbol esters, lipopolysaccharides, or proinflammatory cytokines (50, 53). COX-2, like nitric oxide synthase (37) is thus an inducible enzyme which is produced by cells in response to activation by cytokines or bacterial invasion (53, 53). Although aspirin, indomethacin, naproxen, piroxicam and ibuprofen are predominantly inhibitors of COX-1, diclofenac is approximately equipotent against COX-1 and COX-2 (53-55). Meloxicam, aceclofenac and flosulide, in contrast, are reported to be selective inhibitors of COX-2 at low plasma concentrations (56-58).

Although the selectivity of inhibition of COX-1 and COX-2 by NSAIDs is known to be dependent on the drug concentration, cells, tissues, or source of enzymes used for their evaluation (53, 54), the suppression of PGE_2 production by cells responsible for joint inflammation, at clinical doses that did not give rise to side effects, would appear to represent an important advance in the treatment of OA.

However, several important questions remain unanswered. First, although it is generally assumed that suppression of PGE_2 production by activated macrophages would be a useful pharmacological activity for a drug, it is also known that this prostanoid performs important regulatory functions in these cells. Thus, PGE_2 can exhibit antiinflamma-

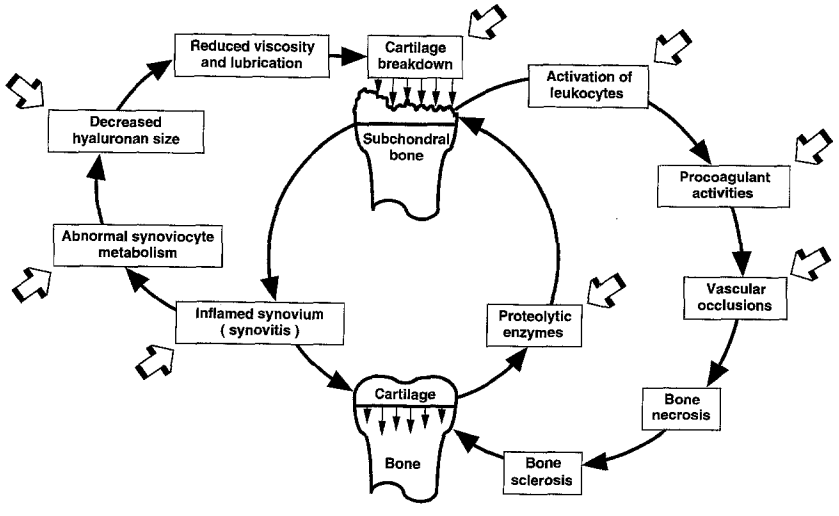

Fig 1. Osteoarthritis results in pathological changes in several joint tissues but most particularly in cartilage, synovium, and subchondral bone. These tissue pathologies are interdependent, and the manner in which they interact is summarized in this figure. The open arrows serve to identify potential sites for pharmacological intervention.

tory effects, such as downregulating the production of inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β]), nitric oxide synthase, and matrix metalloproteinases (MMPs) (59-62). PGE_2 also may elicit an anabolic response by chondrocytes. Di Battista et al (63) observed that when PGE_2 was added to primary cultures of human chondrocytes it induced the autocrine production of insulin-like growth factor-1 (IGF-1), which subsequently led to an increase in synthesis of collagen by these cells. This latter study raises the second question: Do selective COX-2 inhibitors offer any advantages over mixed COX-1/COX-2 inhibitors with regard to their effects on chondrocyte metabolism?

In vitro studies with human chondrocytes (64) have shown that the selective COX-2 inhibitor meloxicam did not suppress PG synthesis when added to the cultures. Conversely, aceclofenac when examined in vitro for its effects on explants of OA cartilage stimulated PG synthesis (65). In a study using a model of spontaneous OA in the rat, Mohr et al (66) noted that meloxicam, at daily oral doses up to 0.8 mg/kg/d administered over 2 years, did not affect the progression of cartilage degeneration and was therefore "chondroneutral." Similar statements have been made for NSAIDs, which are not purported to be selective COX-2 inhibitors (67). However, in most cases this "chondroneutrality" would appear to be dependent on the dosage used (68, 69).

From the preceding discussion, it would appear that NSAIDs with selective COX-2 activity possess the potential to attenuate synovial inflammation at doses that are free of gastrointestinal or renal side effects. However, from the limited information available it has yet to be determined whether these drugs exhibit useful effects on the metabolism of cartilage and bone in OA joints.

The need to develop agents that are capable of ameliorating the symptoms of OA by modifying the underlying pathological condition remains, therefore, an important research objective. A number of drugs have been claimed to fulfill this role, and these have been reviewed in detail elsewhere (70-74). Of these agents, hypersulfated polysaccharides in general and pentosan polysulfate (PPS) in particular have been shown to possess pharmacological activities, which could qualify them as structure-modifying OA drugs (SMOADs) (75). The objective of this review is to examine the data on the known derivatives of PPS with respect to our current knowledge of the pathobiology of OA and to determine whether there is a rational basis for the use of these agents for the treatment of this disorder.

Although the sodium salt of pentosan polysulfate (NaPPS) has been used in Europe for over 40 years as an anti-thrombotic/anti-lipidemic agent, it is only relatively recently that NaPPS and its calcium derivative (CaPPS) have been evaluated as anti-arthritic agents. The calcium derivative, CaPPS, is a new compound that shows many in vitro pharmacological similarities to NaPPS. However, CaPPS exhibits some distinct in vivo activities, including higher oral bioavailability than NaPPS.

Pharmacokinetic studies with tritiated NaPPS in rats (76) and humans (77) have estimated that the bioavailability of the drug when used by the oral route was only 0.5% to 1%. Comparative kinetic studies of blood levels of NaPPS and CaPPS achieved in rats after oral administration, using prothrombin time as a bioassay (78), have indicated that the calcium derivative possessed 10-20% higher oral absorption than the sodium salt. These findings were later confirmed in rats using fibrinolytic assays (79, 80) and in rabbits using an ELISA and a monoclonal antibody directed against PPS (81). There is also some preliminary evidence in OA patients that CaPPS is better absorbed than NaPPS by the intramuscular route of administration (82). Because the co-development of the sodium and calcium salts of PPS for OA have progressed in parallel and they possess the same structural backbone, PPS, their pharmacological activities are reviewed together.

CHEMISTRY

Unlike some other anti-arthritic agents, such as sodium hyaluronate (Healon, Kabi-Pharmacia, Sweden; Hylan, Biomatrix Medical, Canada; Artz, Seikogaku, Kogyo, Japan; Hyalgan, Fidia, Italy), glycosaminoglycan sulfate ester (GAGPS) (Arteparon, Luitpold, Germany), and chondroitin sulfate (Condrosulf, IBSA, Switzerland), PPS is not derived from animal or bacterial sources and is not therefore a potential source of contaminating prion proteins, proteins, or phospholipids. The structure of the PPS backbone of NaPPS and CaPPS is a hemi-cellulose isolated from the wood of the beech

tree (*Fagus sylvatica*). It consists on average of repeating units of (1-4)-linked β-D-xylanopyranoses in which α-D-4-methylglucopyranosyluronic acid residues are linked via oxygen to the 2 position of every 10th xylanopyranose unit (Fig 2). Sulfation of the purified hemicellulose results in almost theoretical esterification of the hydroxyl group present (theoretical 2.0 sulfates/monosaccharide, calculated from sulfur analysis 1.8 to 1.9 sulfates/monosaccharide). Proton and carbon-13 magnetic resonance (^{13}C-NMR) spectroscopic analyses of PPS have confirmed the molecular structural unit shown in Figure 2. The weight average molecular weight (M_w) of NaPPS of approximately 5,700 and number average molecular weight (M_n) of approximately 3,800 were determined by light-scattering techniques after careful fractionation of the polymer (83) and indicated a fairly narrow molecular weight distribution (M_w/M_n = 1.59). The high charge density and rod-like conformation of PPS in solution leads to a lower estimation of its M_w by gel exclusion chromatography (approximately 4,800 Daltons) (84) and allow it to compete effectively with endogenous sulfated glycosaminoglycans (GAGs), such as heparin (HEP), heparan sulfate, and dermatan sulfate (DS), for the protein and cellular sites normally occupied by these GAGs. Because GAGs are ubiquitous and play a variety of regulatory roles in biological systems, it is not surprising that PPS exhibits diverse pharmacological activities. Its smaller molecular domain and unique molecular structure also confers specificity of action not exhibited by the larger naturally occurring GAGs.

THE EFFECTS OF PENTOSAN POLYSULFATE ON CHONDROCYTES AND THE METABOLISM OF ARTICULAR CARTILAGE

Articular cartilage consists essentially of chondrocytes embedded in a hydrated gel of PGs entrapped within a fibrous collagen network. This collagen network serves to resist shearing stresses but also limits the hydration of the PGs entrapped within it. The ability of cartilage to recover after compressive deformation (resilience) is due to the strong water-binding properties (osmotic pressure) conferred by the polyanionic PG molecules present (85). The large (monomer M_w 1 to 2 × 10^6 Da) chondroitin sulfate–rich PG of the cartilage matrix forms macromolecular aggregates with HY and is known as aggrecan. These molecules are the most abundant PG species (in terms of mass) in cartilage. However, cartilage and other connective tissues also contain smaller PGs (decorin, biglycan, fibromodulin) as well as noncollagenous proteins, which perform important regulatory and structural roles within the extracellular matrix (ECM).

Two small DS-containing PGs (DS-PGs) belonging to a family of proteins with leucine-rich repeat regions have been isolated from cartilage (86). Decorin consists of a core-protein of Mr 36,500 and one glycosaminoglycan side-chains containing both DS and CS. The biological functions of these small PG species is still the subject of investigation, but it has been shown that decorin is associated with the 'd' band of collagen fibrils and inhibits fibrillogenesis in vitro (86, 87). Biglycan has a pericellular distribution, and it inhibits attachment to, and

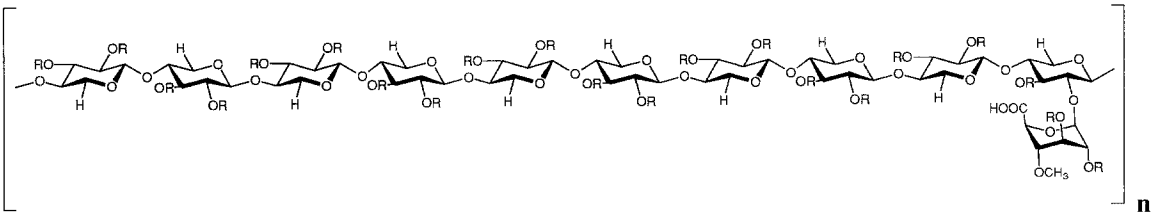

Fig 2. Structural formula of pentosan polysulfate (PPS) isolated from beechwood hemicellulose (*Fagus silvatica*). This formula show that the linear xylan (pentosan) backbone of pentosan polysulfate contains on average one 4-O-methyl-glucuronate side chain linked to the 2 position on every 10th xylose (pentose) ring. The sodium salt of PPS (NaPPS) occurs when R = $SO_3^-Na^+$, whereas the calcium derivative (CaPPS) is when R = $SO_3^-Ca^+_{1/2}$. NMR spectroscopy shows that the individual pentosan rings of PPS are staggered with respect to each other due to electrostatic and steric repulsions of the sulfate groups.

spreading of, fibroblasts (3T3 cells) over a fibronectin-coated matrix (88, 89). There is accumulating evidence to support the view that some of the functions of biglycan and decorin in connective tissues are to modulate cell adhesion, differentiation, and collagen fibrillogenesis. Furthermore, the levels of decorin relative to collagen increase in connective tissue remodeling (90, 91) and in joint cartilage regions subjected to high mechanical loading (92, 93), suggesting that this PG species may contribute to structural reorganization of the extracellular matrix (ECM) in response to altered mechanical stresses.

In normal cartilage, chondrocytes accommodate the mechanical and chemical demands placed on them by synthesizing an ECM most suited to withstand these demands. This biosynthetic activity is in equilibrium with the catabolism of the matrix components mediated by proteinases, whose production, activation, and inhibition are carefully regulated by the chondrocyte.

In OA, there is a net loss of PGs and collagen from the cartilage ECM. The mechanisms responsible have been extensively reviewed elsewhere (31, 94-99). However, it is apparent that the MMPs occupy a central role in this process. Most members of the matrix metalloproteinase family (MMP-1 to MMP-13) degrade aggrecan (100). The site of proteolytic cleavage is the amide linkage between amino acids 341 and 342 (human sequence enumeration) of the interglobular domain (IGD) of the core protein (99, 101, 102), thereby producing two main fragments (Fig 3). The fragment containing the G1 domain remains attached to hyaluronan within the tissue and its C-terminal amino acid sequence . . . DIPEN remains exposed. The other fragment, which diffuses from the cartilage matrix, contains the new N-terminal sequence FFGVF . . . (Fig 3).

Addition of retinoic acid or interleukin-1β (IL-1β) to cartilage explant cultures stimulates the breakdown of aggrecan (and other matrix proteins) and the accumulation of aggrecan degradation products in both the matrix and culture medium (103, 104). When the identities of these proteoglycan fragments were studied by amino acid sequencing, it became evident that the primary aggrecan degradation product released into the culture media contained the N-terminal amino acid sequence ARGSV . . ., which as noted was not that generated by the proteolytic action of any of the MMPs 1 through 13 (103, 105). These same proteolytic fragments were also released into synovial fluid from joints of OA patients (105, 106), confirming

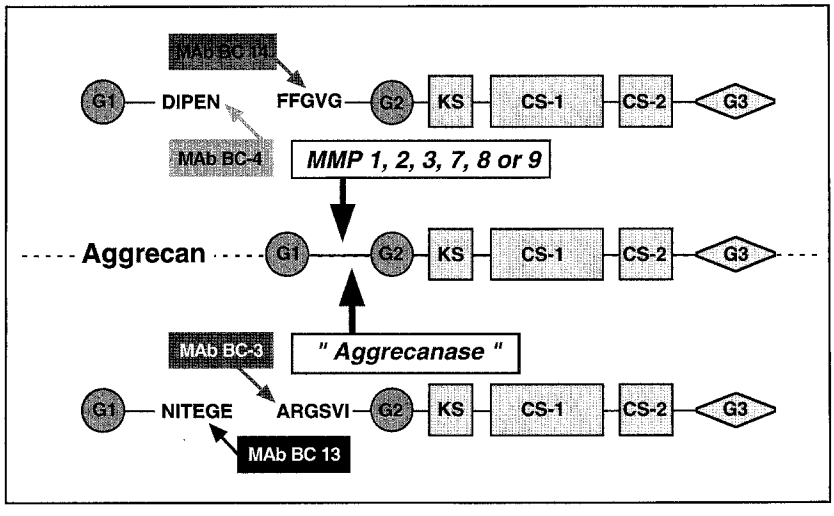

Fig 3. Diagrammatic representation of the structural regions of the PG, aggrecan, showing the sites cleaved by matrix metalloproteinases and the putative "aggrecanase." Terminal amino acid sequences generated by proteolytic cleavage by MMPs and aggrecanase can be identified with specific monoclonal antibodies, the identity of which are also shown. MMP, matrix metalloproteinase; IGD, interglobular domain; G1, globular domain 1; KS, keratan sulfate region; G2, globular domain 2; CS-1, chondroitin sulfate region 1; G3 = globular domain 3; CS-2 = chondroitin sulfate region 2.

that the same cleavage occurred in vivo. Although this discovery was made in the early 1990s, the identity of the enzyme responsible for the specific cleavage of the aggrecan IGD (termed "aggrecanase") has not been unequivocally established. However, it is possible that aggrecanase is a member of the MMP family, because MMP inhibitors can block the release of the core-protein cleavage products from explant cultures (107). Furthermore, a recent report (102) suggests that MMP and aggrecanase cleavage of the IGD are mutually exclusive, supporting the concept of two distinct pathways for cytokine-mediated catabolism of aggrecan. It was suggested that the pathway used may depend on the pool of aggrecan molecules available or differences in "spatial and temporal expression" of the proteinase and their respective endogeneous inhibitors. The problem is compounded by recent evidence indicating that a dual adhesion-MMP protein (ADAM-10) is highly expressed in regions of OA cartilage where PGs are depleted, and this can generate aggrecanase fragments (108). In addition, a hemorrhagic metalloproteinase (Atrolysin C), also a member of the adhesion and metalloproteinase (ADAM) family, has been shown to cleave aggrecan at both the MMP and aggrecanase-susceptible sites in the IGD region (109). The membrane-bound matrix metalloproteinase (MT-MMP) has also been claimed to cleave the PG-IGD at the aggrecanase site (110, 111).

It is evident that specific inhibitors of aggrecanase and MMPs could, in principle, provide a method of reducing the loss of PGs from OA cartilage. However, because chondrocyte biosynthetic activities are also aberrant in cartilage of OA joints, consideration also must be given to the effects such enzyme inhibitors might have on the anabolic aspects of chondrocyte metabolism. In this regard, both NaPPS and CaPPS show positive effects on chondrocyte metabolism but also have been shown to inhibit or downregulate MMP and other enzymes implicated in cartilage destruction. These pharmacological activities of NaPPS and CaPPS are summarized later.

IN VITRO EFFECTS OF PPS ON CHONDROCYTE BIOSYNTHESIS OF DNA AND PGs

Monolayer Cultures

Using an in vitro model of lapine articular chondrocyte injury induced by briefly exposing the cells to sodium iodoacetate (an arthrogenic compound) (112), it was reported that NaPPS preserved cellular activity by increasing the incorporation of PGs into the ECM when used at concentrations between 10 and 200 μg/mL (113). NaPPS, but not an analog of half its molecular weight, also reduced the concentration of PGs released into culture media, suggesting that turnover was affected by the drug (113). In contrast to NaPPS, GAGPS, when cultured under the same conditions, failed to promote the accumulation of PG-rich matrix around chondrocytes.

The concentration-dependent effects of anti-arthritic drugs (indomethacin, diclofenac, NaPPS, GAGPS, tiaprofenic acid, and ketoprofen) on the biosynthesis and release into media of PGs was also studied using primary lapine chondrocyte monolayer cultures (114). Of the anti-arthritic drugs examined, only NaPPS and GAGPS consistently stimulated PG synthesis over the concentration range of 0.1 to 10 μg/mL. At media concentrations of 1.0 μg/mL, NaPPS increased PG synthesis by approximately 25% above the non–drug-treated control levels after 2 to 4 days in culture. This concentration of PPS could be exceeded in human plasma 3 hours after an intramuscular dose of 3 mg/kg to OA patients (115); therefore, the concentration of PPS used in these experiments was within the therapeutic range.

The lapine chondrocyte monolayer culture system was also used to compare the relative effects of NaPPS and CaPPS over the concentration range of 0.1 to 20 μg/mL on desoxyribonucleic acid (DNA) and PG synthesis (116). Although both compounds stimulated DNA synthesis at 1.0 μg/mL, the potency of CaPPS was slightly higher than NaPPS and was also maintained at 10 μg/mL and 20 μg/mL (Fig 4). PG synthesis was enhanced also by NaPPS and CaPPS in vitro over the concentration range of 0.1 to 10 μg/mL. Similar studies were undertaken using monolayer cultures of chondrocytes derived from femoral condyles and tibial plateaux of ovine joints (117). After a 6-day culture period, CaPPS (at concentrations between 0.1 and 100 μg/mL) was added to cultures for 48 hours in the presence of $^{35}SO_4^{2-}$ or ^{3}H-methylthymidine to quantitate PG and DNA synthesis, respectively. In chondrocytes derived from the femoral cartilage, a maximum of 41 ± 2% stimulation of DNA synthesis was observed at 30 μg/mL relative to non–drug-treated controls ($P < .005$). However, with chondrocytes

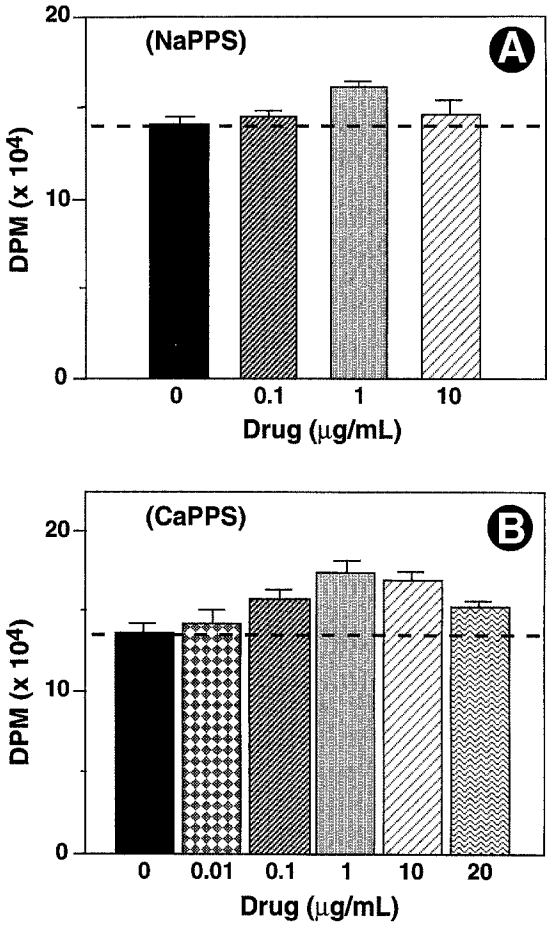

Fig 4. Concentration-dependent effects of NaPPS (A) and CaPPS (B) on DNA synthesis by lapine chondrocytes in monolayer cultures when added to media for 16 hours with ^{3}H-methylthymidine. CaPPS continues to promote DNA synthesis at concentrations (≥10 μg/mL). The corresponding dose of NaPPS does not.

derived from tibial cartilage, a CaPPS concentration of 100 μg/mL decreased DNA synthesis (Fig 5), suggesting reduced cell proliferation in the presence of this high concentration of the drug. Parallel studies showed this not to be the case, because at CaPPS concentrations greater than 10 μg/mL, PG synthesis was found to be higher in tibial chondrocytes than in femoral cells (Fig 6). Kinetic experiments using these ovine cells and ^{3}H-CaPPS showed that tibial chondrocytes maintained in culture at 37°C bound more drug to cell membranes and cytosolic organelles and exhibited a higher cytosolic concentration than the corresponding femoral chondrocytes cultures (Fig 7).

These in vitro studies with ovine chondrocytes were consistent with our previous observation (93) that topographic differences in cartilage metabolism were retained by chondrocytes when the cells are removed from their ECM and established in alginate bead culture. In the absence of CaPPS, femoral chondrocytes were observed to exhibit a higher metabolic activity than tibial cells. In the joint, tibial cartilage is subjected to higher mechanical loading, particularly in the central region not protected by the menisci, than the cartilage of the femoral condyle. The finding that tibial chondrocytes bound more CaPPS and appeared to respond more strongly to CaPPS suggests the presence on these cells of higher number of receptors that bind the drug. This differential response of femoral and tibial chondrocytes could possibly arise as a consequence of the different mechanical demands to which they are subjected within the joint.

Agarose Gel Chondrocyte Cultures

Chondrocytes derived from bovine metacarpophalangeal joints grown in agarose culture according to the method of Aydelotte and Kuettner (118) were used to evaluate the ability of NaPPS, tenidap, RO 31-9790, and diacerhein to stimulate PG synthesis in the absence and presence of IL-1β (119). In the absence of IL-1β, only NaPPS stimulated PG synthesis, whereas in the presence of this cytokine NaPPS, tenidap, and RO 31-9790 were all active.

The proportion of aggrecan monomers isolated from joint cartilage that can aggregate with HY in vitro and the number of monomers per aggregate declines with increasing age (120). Such studies suggest that the decreased aggregation may be partly associated with a decline in synthesis of link protein with age (120). However, the length of the HY chain available for binding of aggrecan monomer units also may be a determining factor.

Verbruggen et al (121) isolated chondrocytes from the femoral condyles of postmortem human donors of various ages and initiated cultures in agarose gels. The total $^{35}SO_4$-incorporation into PGs (aggrecans) were determined and expressed as picograms of $^{35}SO_4$ incorporated into PGs per 10^6 chondrocytes per hour. The total incorporation rates of $^{35}SO_4$ into PGs correlated with the age of the donor tissue. Moreover, the aggrecans that accumulated during culture, when liberated by

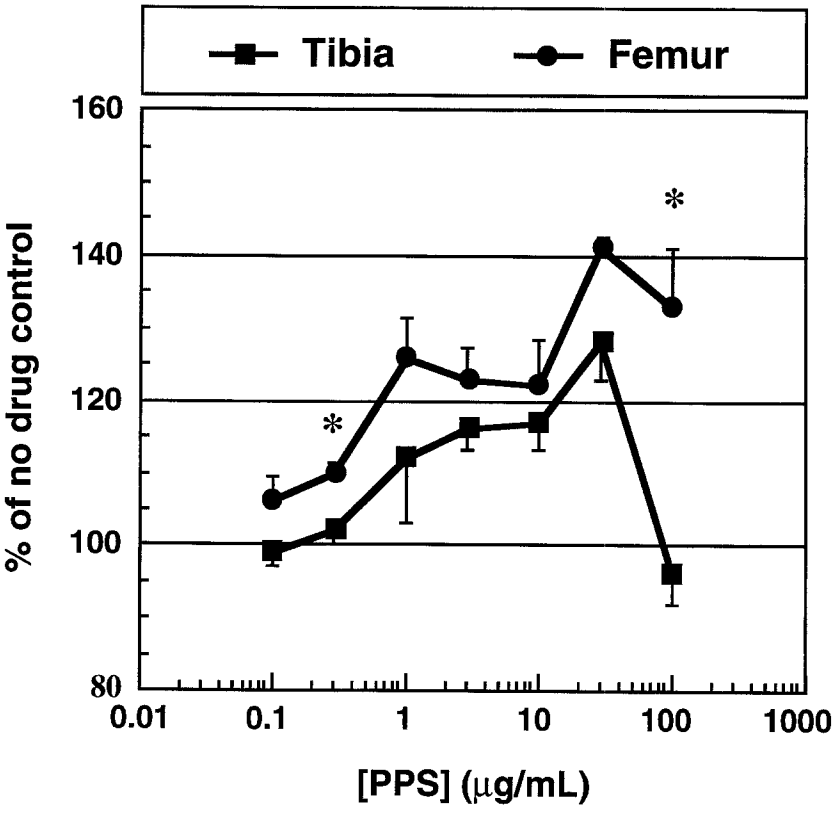

Fig 5. Effect of CaPPS (batch no. A275996) on DNA synthesis by sheep tibial and femoral articular chondrocytes in monolayer culture. Data are expressed as increase of synthesis, as a percentage of non–drug-treated control cultures. Note that at high concentrations of CaPPS, femoral chondrocytes are less responsive to the drug than cells derived from the tibial plateau cartilage. * = $P < .05$ between joint regions.

agarase digestion of the agarose matrix and studied by electron microscopy, were observed as either free monomers or attached to HY in the form of aggregates.

In these aggregates, no free binding sites on the HY chain were found, and the average distance between the aggrecan monomers was 27 nm. Consequently, the length of the HY chain was considered to determine the molecular size of the aggregate formed. Although the average aggregate consisted of 12 aggrecan monomers, mature human articular cartilage cells were also found to synthesize another population of larger PG "super" aggregates with more than 30 aggrecans attached to a single HY chain. The proportions of these super aggrecan aggregates decreased with increasing age of the donor cartilage from which the chondrocytes were derived.

From these studies, it was concluded that the declining synthetic rates of aggrecans, as well as their inability to form "super" aggregates, reflected a failure of the repair capacity of human chondrocytes with increased age (121). It was further speculated that this observation may account for the increasing incidence of OA with aging in the general population. Using this same culture system, this research group examined the effects of HEP, CaPPS, and chondroitin polysulfate (CPS) on the ability of human chondrocytes to synthesize PG aggregates (122). After 2 weeks of culture, the media were supplemented for 1 week with either 10 μg/mL HEP, CPS, or CaPPS. Radiolabeled sulfate ($^{35}SO^4$) was added during the last 24 hours of culture and $^{35}SO_4$ incorporation into aggrecans determined (121). CPS and CaPPS, but not HEP, significantly increased total $^{35}SO_4$ incorporation into PGs. The aggrecans that accumulated during culture, when examined by electron microscopy, showed that CaPPS and CPS but not HEP significantly improved aggrecan aggregate size. For each 100 aggregates observed, the average number of aggrecan monomers attached to HY for the control (no drug), CaPPS, CPS, and HEP were 1,112 ± 18, 1,686 ± 180, 1,278 ± 17 and 1,193 ± 130, respectively. The numbers of aggrecan monomers immobilized on large aggregates (>20 monomers per aggregate out of 100 aggregates observed) are shown in Figure 8. These studies confirmed that CaPPS was capable of increasing the rate of synthesis of PGs in human chondrocytes but also

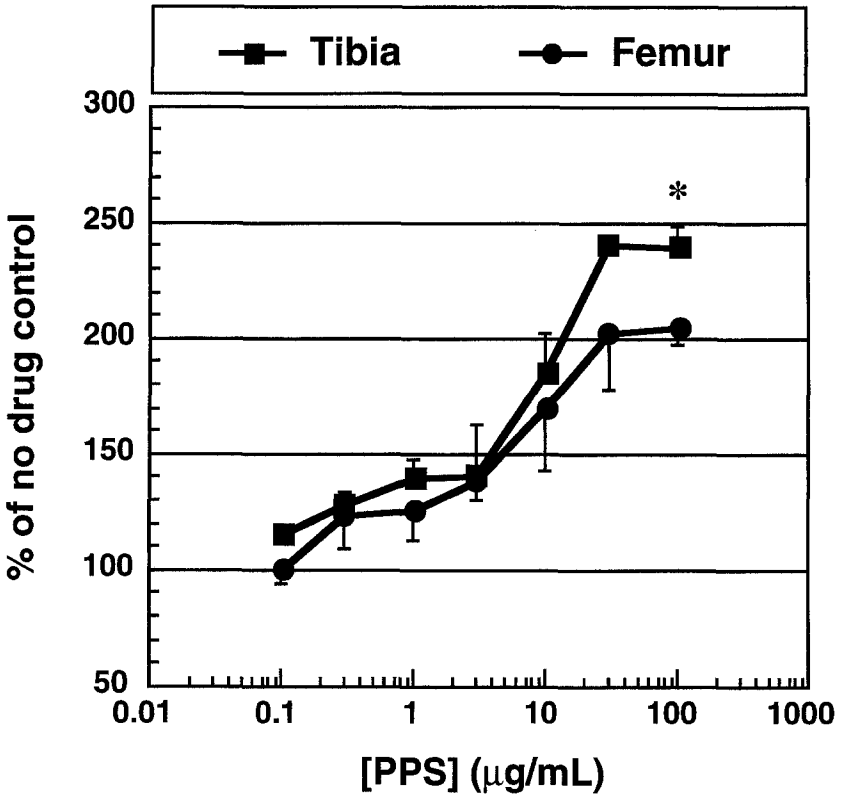

Fig 6. Effect of CaPPS (batch no. A275996) on ^{35}S-incorporation into cell-associated PGs by sheep tibial and femoral articular chondrocytes in monolayer culture. Data are expressed as increase of synthesis, as a percentage of non–drug-treated control cultures. Again, tibial chondrocytes were more responsive than femoral chondrocytes at high media concentrations of CaPPS. * = $P <$ .05 between joint regions.

resulted in the production of large-molecular-weight PG aggregates by these cells.

It was concluded that in an osteoarthritic joint, where PG synthesis and matrix integrity are reduced, such metabolic effect of CaPPS may lead to the accumulation of large PG aggregates in the cartilage matrix, thus improving its biomechanical properties.

IN VITRO INHIBITORY EFFECTS OF NaPPS AND CaPPS ON ENZYMES IMPLICATED IN CARTILAGE CATABOLISM IN OA

The proteinases responsible for the catabolism of components of the cartilage ECM in OA joints have been the subject of investigation for many years. The controversy concerning which proteinases cleave the G1-G2 domain of aggrecan has already been discussed here in some detail. However, a number of other sites on aggrecan, as well as on other species of PGs, are susceptible to enzymatic attack, for example, the E_2 region of aggrecan (Fig 3) and the GAG chains themselves. Furthermore, the most abundant structural proteins of cartilage, types II, IX, and XI collagen, all have been shown to be degraded in OA tissues and some of the proteinases responsible identified (31, 123-126). Fibrillar collagens are cleaved in the helical regions by true collagenases, which belong to the matrix metalloproteinase family (MMP-1, MMP-13). Denatured collagen (gelatin) can be degraded by gelatinase (MMP-2, MMP-9) and by many other proteinases, including trypsin, human granulocyte elastase (HGE), and cathepsin-G (127). HGE also will cleave native fibrillar collagens in the teleopeptide region (128) as well as the minor collagens (types IX, X, XI) and, like other serine proteinases (plasmin, trypsin), will activate many of the proforms of the MMPs (100, 129).

The definition of a proteinase, in terms of the substrate it cleaves, thus has little meaning today, because most of those enzymes present in cartilage show a wide range of specificities, some which are summarized in Table 1.

NaPPS and CaPPS are strong inhibitors of many of these proteinases, and modulate their production by chondrocytes and other cells. In addition, NaPPS attenuates glycosidases (130, 131) and heparanase activities (132), both of which play important roles in the turnover of the GAGs responsible for binding growth factors (eg, fibroblast growth factors) and

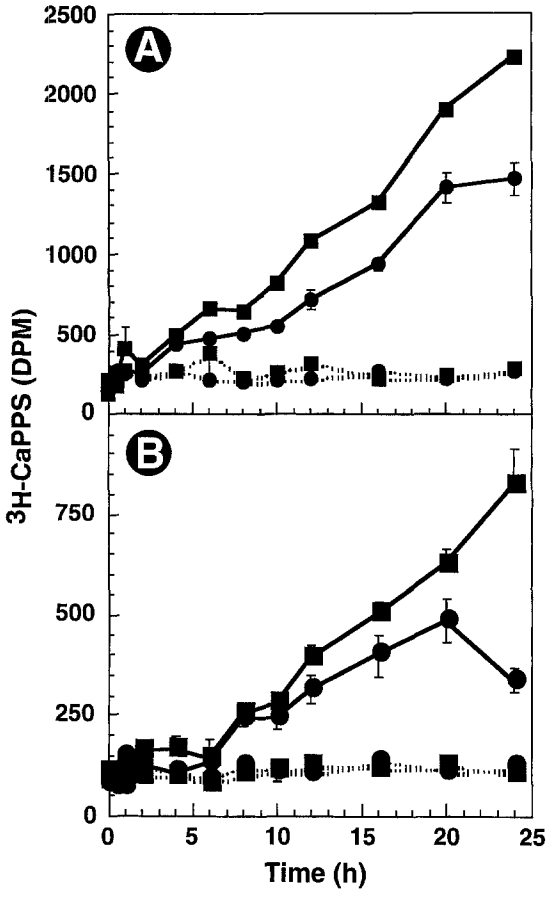

Fig 7. Uptake of ^{3}H-CaPPS by tibial (■) and femoral (●) ovine articular chondrocytes at 37°C (solid line) or 4°C (dotted line). (A) ^{3}H-CaPPS in the cytosol; (B) ^{3}H-CaPPS associated with the cell pellet. Tibial chondrocytes showed a higher uptake of ^{3}H-CaPPS than the corresponding femoral cells.

regulating cellular division and migration. NaPPS also is a potent inhibitor of intracellular kinases such as protein kinase C (133). Protein kinase C and other protein kinases are intracellular signaling proteins that transduce, by protein phosphorylation, the IL-1–mediated gene expression of MMPs (134, 135), tissue inhibitor of matrix metalloproteinase (TIMP) (135), and PGE_2 (136) by chondrocytes and other cells.

PPS, as the sodium or calcium salts, is a potent inhibitor of serine proteinases, including HGE (137-140) and cathepsin G (141). Typical HGE inhibition curves for NaPPS and CaPPS are shown in Figure 9. Kinetic studies reported by Baici et al (140) have shown that NaPPS behaves as a simple hyperbolic, noncompetitive inhibitor, the enzyme-inhibitor interaction being electrostatic in nature. Barg et al (142) proposed that NaPPS interacted with the natural substrates of HGE to form complexes that were then resistant to degradation by the enzyme. Andrews et al (139) tested this hypothesis using an anion-exchange (DE52) competitive binding assay. This method showed that the binding of NaPPS to HGE was very much greater than binding to elastase substrates such as gelatin or PGs.

These findings support the concept of a specific electrostatic interaction between PPS and HGE. Some insight into the nature of the PPS interactions with cationic proteins, such as HGE, was provided by Lentini et al (143), using ^{13}C-NMR techniques. From chemical shift and relaxation time measurements, the stoichiometry of binding of NaPPS to a purified gelatin fraction of average $M_w = 5 \times 10^5$ Da was found to be 2:1 (PPS:gelatin). The association constant for the complex was assessed to be 10^4 to 10^5 M^{-1}. Using molecular space-filling models of these structures, it was shown that the lysine-75 and arginine-78 residues present on the α-chains of gelatin could favorably interact electrostatically with sulfate ester groups in the 2 and 5 positions of the PPS xylopyranose rings. Because the sulfated xylopyranose rings of PPS are staggered by 180°C due to steric repulsions (Fig 2), the polypeptide sequence also could be duplicated on the other flank of the polysaccharide chain, explaining the 2:1 gelatin-to-PPS stoichiometry. Such multisite electrostatic interactions of the sulfate ester groups of PPS with proteins would be supplemented by additional binding of the carboxylic acid group present on the glucuronic acid residues situated along the PPS backbone (Fig 2).

Using bovine nasal cartilage as a substrate, Kruze et al (138) found that NaPPS produced 60% and 18% inhibition of HGE at inhibitor concentrations of 10^{-6} mol/L and 10^{-7} mol/L, respectively. These inhibition values, using cartilage as the substrate, were lower than those obtained with small synthetic substrates, possibly because of nonspecific binding of some of the drug to the cartilage collagens, as shown by the studies of Andrews et al (139) and Lentini et al (143).

From inhibition curves, such as those shown in Figure 9, an IC50 (inhibitor concentration at which 50% inhibition occurs) of approximately 10^{-8} mol/L was determined for NaPPS and CaPPS when

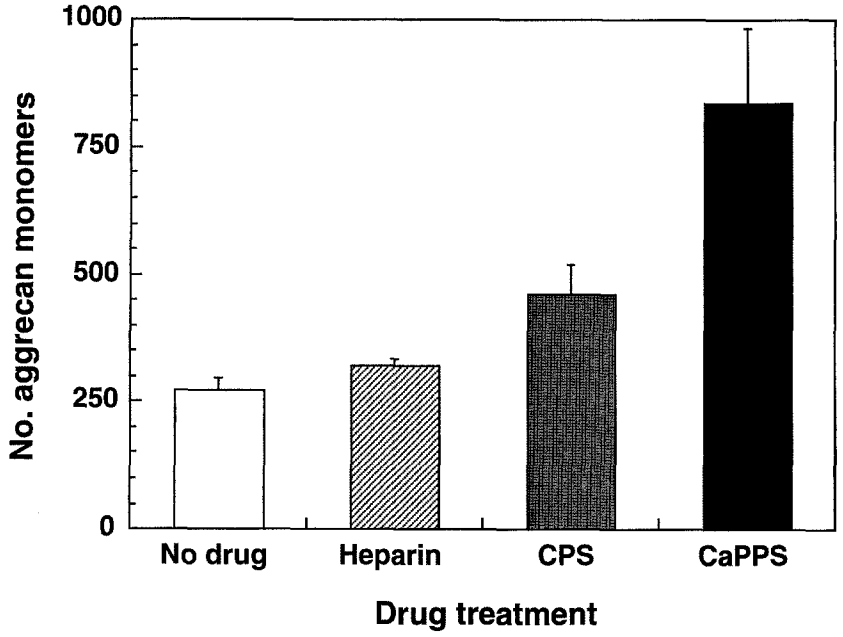

Fig 8. Number of proteoglycan monomers present on large aggregates synthesized by human articular chondrocyte agarose gel cultures in the absence and presence of various polysulfated polysaccharides. (CPS, chondroitin polysulfate) (122). CaPPS produced a significantly higher number of large aggregates than non–drug-treated control cultures ($P < .05$).

the concentration of HGE was 6.5×10^{-9} mol/L and excess amounts of the synthetic substrate succinyl-alanyl-alanyl-valyl-nitroanilide (SAAVN) was used.

These inhibition constants were in good agreement with the results by Baici et al (140), who reported an IC50 of 15×10^{-8} mol/L at a HGE concentration of 131×10^{-9} mol/L. Andrews et al (139) obtained an IC50 of 17×10^{-8} mol/L, but the enzyme concentration was not reported. In contrast to the results reported by Baici et al (140), Andrews et al (139) found the inhibitory activity of NaPPS

Table 1: Some Proteinases Identified in OA Cartilage as Protein or mRNA

Proteinase	Common Substrate(s)	References
Collagenase (MMP-1)	PGs, LP, Collagens II, X	100, 363, 364, 365
Gelatinase A (MMP-2)	PGs, Denatured collagen, Collagens X, XI	100, 366, 367
Stromelysin (MMP-3)	PGs, LP, Collagen II, IX, X, XI	101, 363, 368-370
Neutrophil collagenase (MMP-8)	PGs, Collagen II	364, 371, 372
Gelatinase B (MMP-9)	PGs, Denatured collagen	100, 366, 367
Collagenase-3 (MMP-13)	PGs, Collagen II	373, 374
Membrane type-1 matrix metalloproteinase (MT-1-MMP)	PGs, Aggrecan (activation of pro-MMP-2)	164
Atrolysin C (metalloproteinase HT-D)	Aggrecan	109
ADAM-10	Aggrecan	108
Plasmin	PGs, Fibrin	367, 375
Tissue plasminogen activator	Plasminogen	367, 376, 377
Urokinase-type plasminogen activator	Plasminogen	367, 376, 377
Leukocyte elastase	PG, Collagen I, II, IX, X, XI	378-380
28 kDa Serine proteinase	Aggrecan	381, 382
Calpain	PG	99, 386
Cathepsin B	PG, Collagen II, IX, XI	383-385
Cathepsin D	PG, Denatured collagen	387, 388
Cathepsin G	PG, Collagen II, TIMP	378
Cathepsin L	PG, Denatured collagen	389, 390

Abbreviations: MMP, matrix metalloproteinase; ADAM, A disintegrin and metalloproteinase.

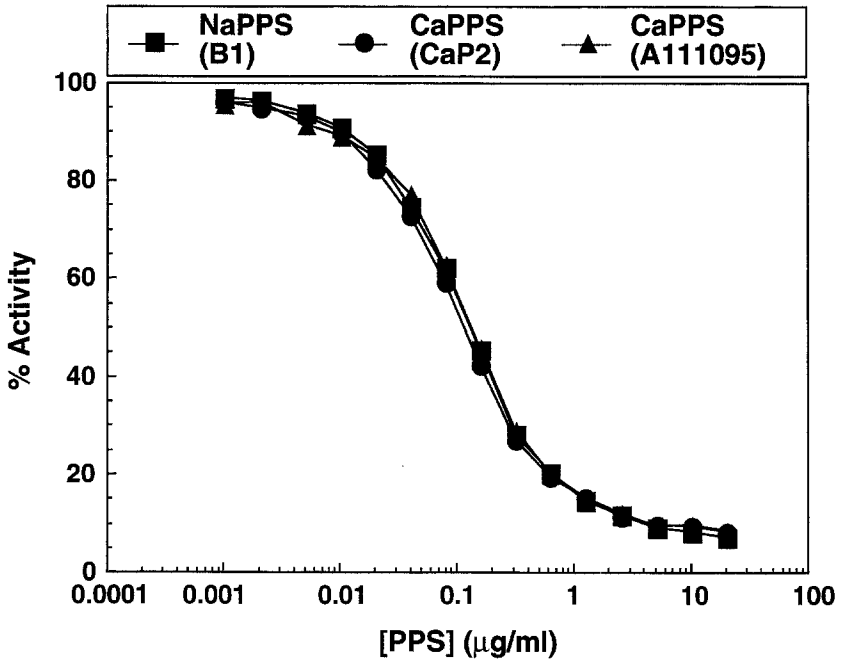

Fig 9. Typical concentration-dependent inhibition of human granulocyte elastase by different batches of NaPPS and CaPPS using the synthetic substrate succinyl-alanyl-alanyl-valyl-nitroanilide (SAAVN) showing similar inhibitor profiles for the drugs.

against HGE could only reach a maximum of 90%, a finding that was compatible with the report of Barg et al (142). The effects of NaPPS and CaPPS on other serine proteinase, including those involved in the complement and hemostatic/fibrinolytic systems, are discussed in a later section.

NaPPS exerts a concentration-dependent inhibition of testicular- and aortic wall–derived hyaluronidases in vitro (131, 144, 145). This inhibitory activity was more potent than that exerted by HEP or DS. Concentrations of 0.5 to 5 μg/mL produced suppression of activity ranging from 65% to 95% (145). More recently the hyaluronidase of human plasma was purified and cloned (146) and also was shown to be strongly inhibited by NaPPS (Stern, personal communication). Because HY is the natural substrate for hyaluronidase and is turned over in both cartilage and synovial fluid (29, 72, 121, 146), inhibition of hyaluronidase by NaPPS or CaPPS could serve to maintain PG aggregation in cartilage and preserve the viscoelasticity of synovial fluid. Platt (148) preincubated NaPPS with explants of human cartilage and showed histochemically, using toluidine blue, that retention of the dye to the matrix was maintained when the samples were incubated with testicular hyaluronidase. Leiss and Kalbhen (149) as well as Kruze and coworkers (138, 150) conducted similar experiments using crude HGE preparations in place of hyaluronidase. NaPPS preserved tissue levels of PGs when the drug was preincubated with the cartilage before adding HGE.

Other lysosomal enzymes inhibited by NaPPS include chondroitin-4-sulfatase and β-acetyl-glucosaminidase (130) and cathepsin B1 (151). Using the synthetic substrate (N-alpha-benzoyl-DL-arginine-z-naphthylamide; BANA), it was reported that NSAIDs, such as aspirin, diclofenac, and indomethacin, only marginally inhibited cathepsin B1 at concentrations of 10^{-5} mol/L, whereas NaPPS exhibited 72% inhibition at 10^{-6} mol/L.

Animal models of arthritis have shown a decrease in stromelysin and collagenase activities and an elevation of their endogenous inhibitor, TIMP, in cartilages of animals administered CaPPS (147) or NaPPS (152, 153), suggesting that these MMPs could also be inhibited directly. However, an in vitro study by Nethery et al (154) reported that although NaPPS inhibited stromelysin, it enhanced collagenase activity. This stimulatory effect of NaPPS on interstitial collagenase (MMP-1) activity could not be confirmed using techniques such as zymographic or Western blotting analysis for MMPs; in fact, NaPPS and CaPPS were found to be inhibitors of collagenase and gelatinase (147). A possible explanation for this discrepancy may be attributed to the technique employed by Nethery et al (154) to determine collagenolytic activity. The

method determined the amount of a type 1-collagen-Coomassie blue complex remaining in culture-plate wells after digestion with the collagenase in the absence or presence of NaPPS. Because NaPPS binds (143) to collagen (gelatin), it could have competed with the dye-binding sites on the collagen substrate, resulting in solubilization of the chromophore and indicating enhanced degradation of collagen. Further studies on the direct effects of CaPPS on the inhibition of various MMPs are in progress in the author's laboratories in an attempt to clarify this issue.

Reports by Rogachefsky et al (152), Grumbles et al (153), and Ghosh et al (147) that activities of MMPs are reduced in cartilages of animal models of OA or in chondrocyte cultures in the presence of NaPPS and CaPPS could suggest that alternative pathways other than direct enzyme inhibition may be influenced by these agents. Chondrocytes isolated from the proliferation-maturation zone of 42-day-old rachitic rat tibia epiphyseal cartilage when grown in serum-free cultures with IL-1β showed enhanced expression of interstitial collagenase mRNA (155). The rat growth plate interstitial collagenase is homologous with human MMP-13 (Table 1) and can degrade native type II collagen and PGs (156). Levels of MMP-13 also are elevated in OA cartilage (125, 126). It had been reported that the upregulation of MMP-13 transcription by IL-1 occurred via the Fos-Jun/AP-1 complex, which binds to cis-acting elements on the promoter of the gene (156). Incorporation of NaPPS (1 to 5 $\times$ 10^{-6} mol/L) into the IL-1β (1 ng/mL)-stimulated chondrocyte cultures decreased the activity of the MMP-13 promoters as shown, using both 256 Kbase and 77 Kbase constructs (Fig 10) (156). As reported by Busch et al (157), the sulfated polysaccharides, such as HEP and NaPPS, can bind to the mesenchymal cells through high-affinity receptors and then become internalized and localize in the nucleus. It was proposed (156) that within the nucleus NaPPS downregulated MMP-13 gene expression by interaction with the transcription factor complex, as shown diagrammatically in Figure 11.

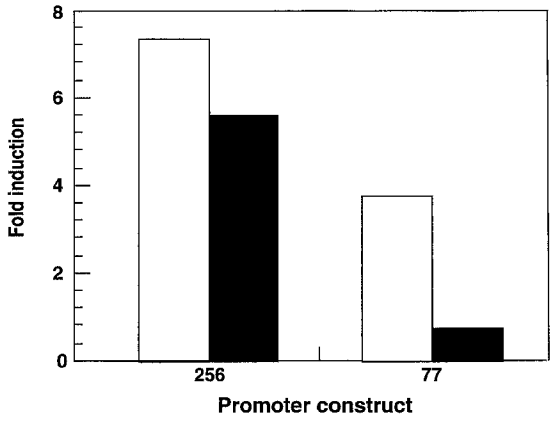

Fig 10. Effects of NaPPS on the activity of the interstitial collagenase (MMP-13) promoter in transient cell transfection experiments using different size promoter constructs. □ = control (no drug); ■ = with NaPPS (155).

IN VIVO EFFECTS OF NaPPS AND CaPPS ON ARTICULAR CARTILAGE IN ANIMAL MODELS OF JOINT ARTHROPATHY

Joint Atrophy in the Rabbit

Using an atrophy model of arthritis induced in rabbits by immobilizing their hind limbs in extension, Golding and Ghosh (158) evaluated the effects of NaPPS on articular cartilage degradation at two different dose levels. In the absence of the drug, immobilization of joints for a period of 4 weeks produced significant changes in the composition of articular cartilage. Both hexuronic acid content (as a marker of PGs) and PG extractability were significantly reduced ($P < .05$) relative to nonimmobilized control cartilages. The contralateral (free) joint of the immobilized group also showed a significant decrease ($P < .05$) in articular cartilage PG content relative to the nonimmobilized control group. The loss of PGs from articular cartilage of immobilized rabbit joints was reduced by intramuscular (IM) administration of NaPPS at 10 mg/kg every 48 hours over the 4-week immobilization period. The cartilage PG content in drug-treated animals was held to within the lower range of the control values, whereas PG extractability was maintained at control cartilage levels. Similar results were obtained after analysis of the cartilages from contralateral joints of drug-treated animals.

After 4-week joint immobilization, the proportion of nonaggregatable PGs extracted from articular cartilages was 60% of the total present, compared with 28% in cartilage of nonimmobilized joints. Similar PG aggregation studies of PGs extracted from articular cartilage from immobilized joints of NaPPS-treated animals showed that this

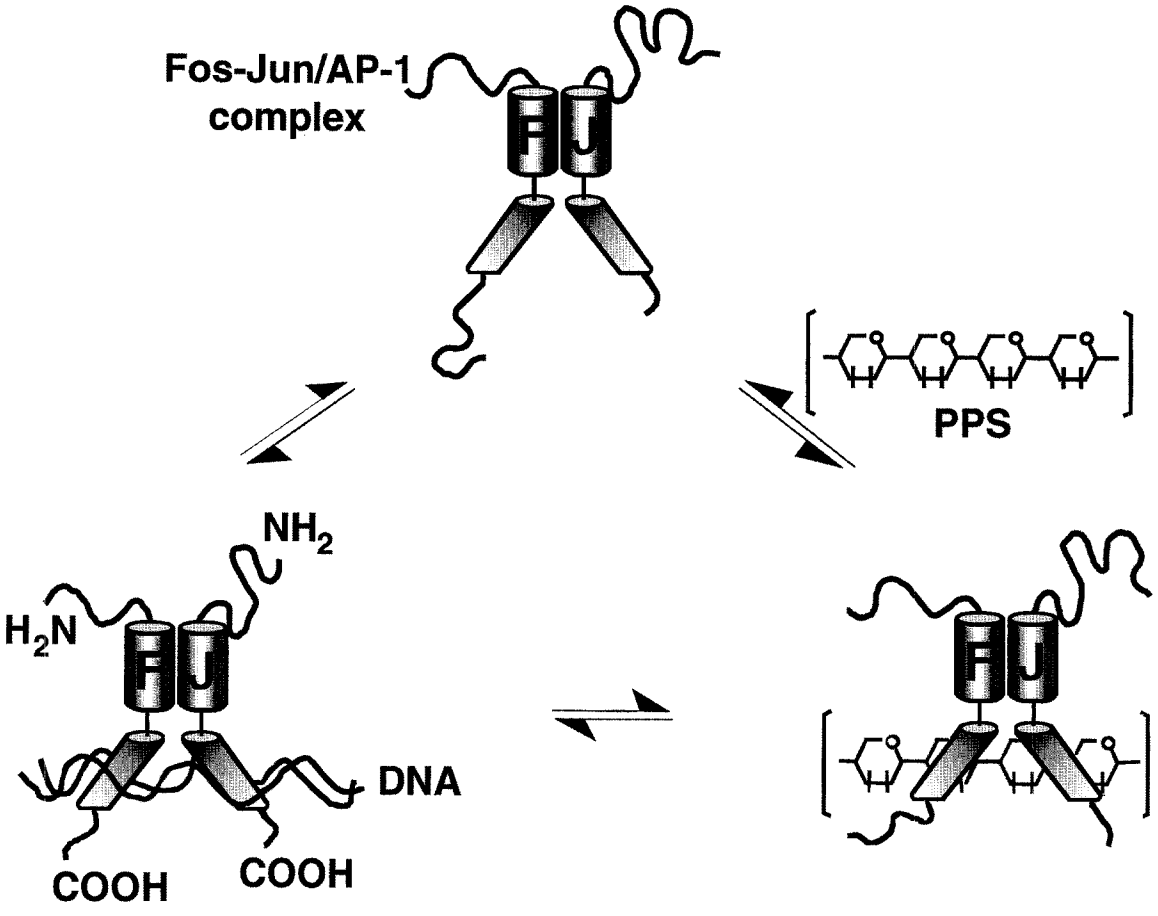

Fig 11. Proposed model for the modulation of MMP-13 gene transcription activity by NaPPS and CaPPS. As reported by Smith and Ghosh (117) and Busch et al (157) CaPPS and NaPPS rapidly bind to mesenchymal cell surface receptors, are internalized, and are transported to the cell nucleus, where they bind to nuclear proteins. The observed downregulation of MMP-13 mRNA by NaPPS in cultured chondrocytes stimulated with IL-1β was found in transfection experiments to arise from the reduction by the drug of ligand-stimulated 12-O-tetradecanoyl phorbol-beta-acetate reactive element (TRE) containing oligonucleotides. It was suggested (156, 157) that NaPPS, like heparin, can interact with the Fos-Jun/AP-1 promoter complex required for gene transcription, thereby attenuating the cellular response to stimuli, such as IL-β. The respective binding constants for NaPPS and CaPPS with the gene promoter complexes have not been determined. (Reprinted from The Journal of Cell Biology, 1992, 116:41, by copyright permission of The Rockefeller University Press [157].)

aggregation deficiency was restored to control levels. These results are summarized in Table 2. When the dosage of NaPPS administered was reduced to 5 mg/kg IM every 48 hours over the same period, the amounts of PGs present in cartilage and their ability to aggregate were indistinguishable from the values obtained for the non–drug-treated immobilized group.

Joint Atrophy in the Canine

A canine model of disuse atrophy induced by nonrigid fixation of a hind limb for 4 weeks was used by Grumbles et al (153) to examine the effects of NaPPS alone or in combination with recombinant human insulin growth factor-1 (rhIGF-1) on cartilage of the immobilized joint. In the non–drug-treated atrophy group, levels of MMPs in cartilage

Table 2: Effects of Pentosan Polysulfate (NaPPS) (10 mg/kg IM Every 48 Hours) on Articular Cartilage (AC) From Control (Nonimmobilized) Rabbit Joints and Joints Subjected to Immobilization in Extension for 4 Weeks

	Control Joint AC† (n = 4)	Contralateral Joint AC (n = 4)		Immobilized Joint AC (n = 4)	
	Non–Drug-Treated Group	Non–Drug-Treated Group	Drug-Treated Group	Non–Drug-Treated Group	Drug-Treated Group
Collagen (μg/mg* ± SEM)	530.7 ± 40.0	640.2 ± 57.6	714 ± 1.1	676.6 ± 51.1	708.2 ± 15.7
Total uronic acid (μg/mg* ± SEM)	40.7 ± 9.2	27.2 ± 3.8	29.7 ± 3.8	14.2 ± 0.8	29.6 ± 1.2
Uronic acid remaining in extracted tissue (μg/mg* ± SEM)	4.8 ± 0.5	5.5 ± 0.1	7.5 ± 1.8	3.6 ± 0.1	6.3 ± 0.4
% Extractability ± (SEM [4 mol/L GuHCl])	87.9 ± 3.2	79.5 ± 4.5	90.5 ± 0.6	74.6 ± 1.5	86.8 ± 1.2
% Nonaggregatable proteoglycans in extracts ± SEM	28.2 ± 6.2	32.2 ± 8.6	32.4 ± 6.9	59.6 ± 2.0	29.6 ± 4.3

Abbreviations: n, number of animals in each group; SEM, standard error of the mean.
*Dry weight cartilage.
†From nonimmobilized rabbit joints.
Modified from Golding and Ghosh (158).

were significantly elevated and TIMP reduced relative to nonimmobilized controls ($P < .05$). The combined treatment of intraarticular (IA) rhIGF-1 (1 μg, 3 × weekly) with NaPPS (2 mg/kg subcutaneously once weekly for 2 weeks) partially corrected this imbalance and reduced the loss of PGs from articular cartilage ECM.

Sodium Iodo-acetate–Induced Degeneration in Knee Joint Articular Cartilage

Kalbhen and Blum (159) used the sodium iodo-acetate–induced arthropathy model in chickens to evaluate the protective effects of NaPPS on cartilage as assessed radiologically and histologically. It was found that weekly IA injections of 1 to 5 μg of NaPPS over a 6-week period abrogated the degenerative changes caused by iodo-acetate injection. When NaPPS was injected into normal joints, it failed to elicit cartilage injury. This result contrasted with IA injections of 1 to 5 μg NSAIDs, such as phenylbutazone or indomethacin, which caused significant damage to joint cartilage.

Lapine Model of Osteoarthritis Induced by Meniscectomy and Colateral Ligament Transection

Howell et al (160) used the Moskowitz rabbit OA model (161) to evaluate the effects of anti-arthritic drugs on cartilage breakdown. In this model, NaPPS and Arteparon (GAGPS) at 1 mg/kg were administered intraarticularly twice weekly to groups of rabbits in which medial partial meniscectomy had been performed. An equivalent dose of HEP was given intraarticularly to meniscectomized rabbits as a control. One week postmeniscectomy, animals in the experimental groups were administered these agents for a further 11 weeks. Histological sections were evaluated with respect to the severity of cartilage damage using the Mankin scoring system (162). The mean histological Mankin scale grade for the controls was 10.33 ± 1.21 (moderately severe). A reduction in the scoring to 3.2 ± 1.2 was observed in the animals who received NaPPS, which was highly significant ($P < .001$). In contrast, HEP failed to reduce the Mankin score ($P > .1$). The aggregational properties of PGs extracted from the cartilages of meniscectomized controls who received saline were poor as assessed by transport ultracentrifugation. Treatment with GAGPS or PPS improved aggregation, but the change was not significant, although cartilage PG content was maintained at control levels.

An Ovine OA Model Induced by Meniscectomy

In an ovine model of OA induced by meniscectomy, a once-weekly IA injection of NaPPS (25 mg) for 4 weeks into the stifle joint, beginning 16 weeks after surgery, improved joint function and indices of pathological change, whereas corresponding HY treatment was less effective (163). Force

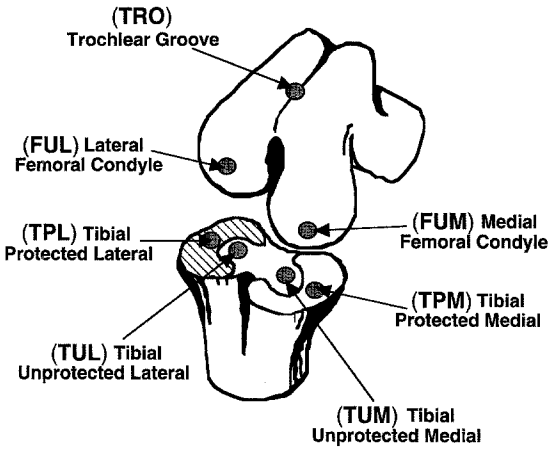

Fig 12. Schematic representation of laterally meniscectomized ovine joint showing cartilage sites (●) sampled for analysis of MMPs. The hatched area on the lateral tibial plateau (TPL) shows the area normally covered by the lateral meniscus.

plate studies indicated that NaPPS improved gait and radiological scores were lower than non–drug-treated saline-injected OA controls ($P < .05$). The Mankin (162) histological scores for articular cartilage damage of the femoral condyle in the meniscectomized compartment were reduced for both PPS- and HY-treated animals ($P < .05$). In a later study (147) using bilateral meniscectomy in sheep and subcutaneously administered CaPPS (3 mg/kg) given for 5 weeks after OA had been established for 4 months, the levels of cartilage MMPs were decreased. Different topographical regions of joint cartilage (Fig 12) were analyzed for MMP and TIMP activity using zymographic and Western blotting techniques. In CaPPS-treated animals, MMP activity was reduced in cartilage of the meniscectomized compartment relative to controls. The results obtained for MMP-2 and MMP-3 are shown in Figure 13. MMP-2 is a proteinase that can degrade a variety of matrix components, including PGs, link protein, and collagens. It is elevated in OA cartilage (97, 96, 126, 164) and is probably converted from the proform into the active species by membrane-type I matrix metalloproteinase (MT1-MMP), an enzyme that is localized to chondrocytes in the superficial and transitional zone, where cartilage degradation is highest in OA (164).

Hydrocortisone-Induced Cartilage Atrophy Model in the Rabbit

IA administration of high-dose corticosteroids into joints of mature New Zealand rabbits induces arthropathy and the loss of PGs from articular cartilage (165, 166). This model was used to evaluate the ability of NaPPS to preserve chondrocytes anabolic activities against the suppressive

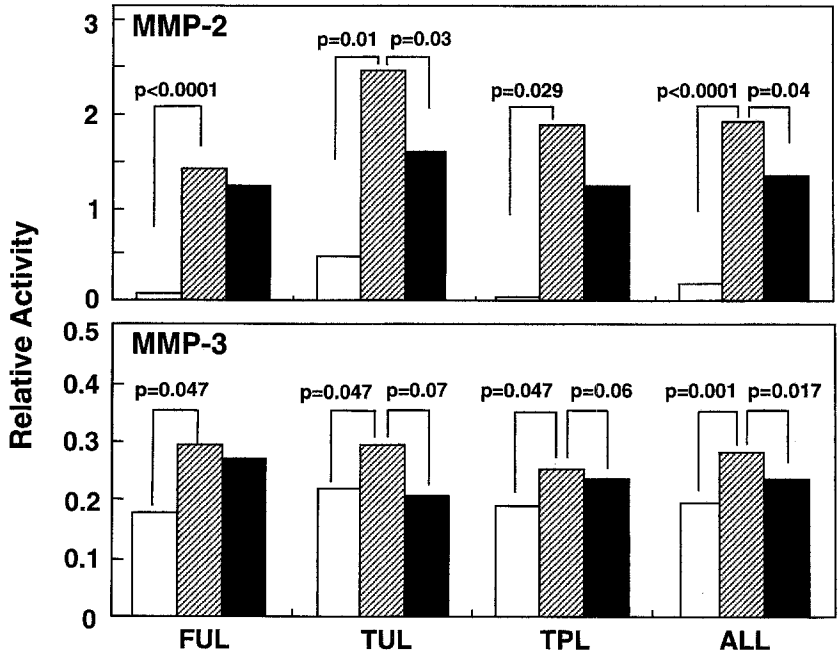

Fig 13. Levels of the activity of matrix metalloproteinases MMP-2 and MMP-3 in cartilage samples taken from the FUL, TUL, TPL regions (see Fig 12) of unoperated control (□); OA (▨) and OA + CaPPS-treated (■) animals. Although individual regions showed significant differences, greater significance was obtained by combining the data for all joint regions (ALL) ($P < .05$ was considered significant).

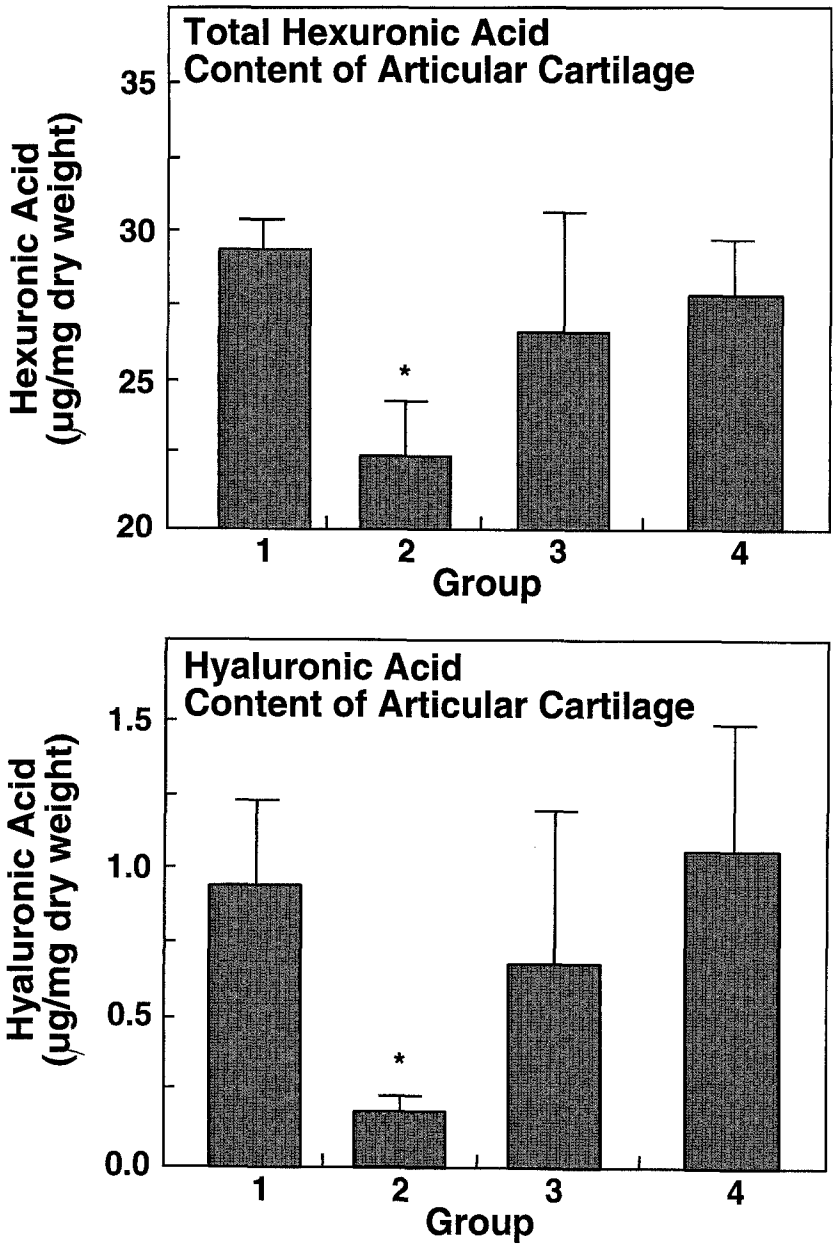

Fig 14. The in vivo suppressive effect of hydrocortisone (HC) on rabbit articular cartilage metabolism can be partially abrogated by NaPPS. Hyaluronan (hyaluronic acid) (HY) and PG content (as measured by hexuronic acid) of cartilages of animals administered 8-weekly injections of HC (group 2) were depressed relative to joints receiving saline (group 1) (*$P < .05$). NaPPS alone (group 4) did not decrease PG synthesis, but when used in combination with HC (group 3), it maintained PG and HY levels in joint cartilages to within control (saline) levels. (Data from Kongtawelert et al [167].)

effects of hydrocortisone succinate (167, 168). HC (25 mg) was administered intraarticularly, once a week for 8 weeks, to a group of mature New Zealand white rabbits, whereas in other groups, joints were injected intraarticularly for the same period with either NaPPS alone (5.0 mg) or a mixture of NaPPS (5.0 mg) and HC (25 mg). As is evident from Figure 14, NaPPS when administered with HC abrogated the ability of steroids to induce the loss of PGs from joint articular cartilage. Significantly, cartilage HY levels, which were diminished in the HC-treated group, were also restored in the NaPPS/HC-treated group, to control values. The incorporation of $^{35}SO_4^{2-}$ into joint articular cartilage, as shown by the determination of its specific activity, was also normalized by NaPPS treatment. Furthermore, in the NaPPS/HC-treated group, serum levels of keratan sulfate (KS), a putative marker of cartilage PG turnover, was also normalized.

Table 3: Mean Proteoglycan Contents (as Hexuronic Acid μg/mg Dry Weight Cartilage) of Individual Rabbit Articular Cartilages 7 Days After Implantation Into Peptone-Inflamed Subcutaneous Air Pouches of Rats Receiving Various Doses of Drugs

Drug	Dose (mg/kg)			
	50	20	10	0
Arteparon	14.7	15.7	7.3	16.7
	15.7	12.2	10.2	10.5
	27.4	12.7	18.9	14.4
	30.0	15.4	17.0	15.0
	12.9	11.9	20.8	12.4
			16.1	
Mean ± SD Hexuronate	20.1 ± 7.9	13.6 ± 1.8	15.1 ± 5.2	13.8 ± 2.4
NaPPS	27.4	12.2	16.1	12.2
	22.2	10.2	17.3	11.7
	26.2	13.0	21.8	14.4
	29.9	16.5	24.6	15.0
	22.1	12.3	22.4	13.5
	24.1	14.6		
Mean ± SD Hexuronate	25.3 ± 3.1*	13.1 ± 2.2	20.4 ± 3.6*	13.4 ± 1.4
DH-40J (ZnPPS)	23.9	18.2	22.6	14.8
	21.2	17.2	17.3	10.2
	24.9	22.7	14.7	15.7
	24.0	24.8	20.8	12.3
	21.8	19.6	13.8	14.1
	20.3		23.1	
Mean ± SD Hexuronate	22.7 ± 1.8*	20.5 ± 3.2	18.7 ± 4.0	13.4 ± 2.2

NOTE. The animals were injected intramuscularly with Arteparon, NaPPS, or DH-40J (ZnPPS) at the doses shown. Results are expressed as mean ± standard deviation.

Abbreviation: SD, standard deviation.

*Significantly different from non–drug-treated controls ($P < .05$).

Modified from Francis (173).

Cartilage Implanted Into Inflamed Subcutaneous Air Pouches in Rats

The subcutaneous air-pouch cartilage model used in laboratory rodents (169-171) has been employed to study the effects of NSAIDs and other drugs on inflammatory cell-cartilage interactions. In the studies described by Francis et al (172, 173), rabbit articular cartilage was implanted into 7-day-old subcutaneous air pouches established beneath the dorsal fascia of mature rats. The injection of a 3% (weight/volume) peptone solution into the pouches stimulated an acute inflammatory reaction with the influx of large numbers of neutrophils and the concomitant loss of PGs from the cartilage implant. Using this model, it was shown that NaPPS when injected into the pouch or intramuscularly was superior to two other sulfated polysaccharides, Arteparon and DH-40J (ZnPPS), in retarding the loss of PGs from implanted articular cartilage (Table 3). Moreover, in cartilages of the NaPPS-treated animals, the mean level of PG aggregation was higher than the GAGPS and non–drug-treated control groups (172). In a parallel investigation using the same rodent model (174), the injection of NaPPS (2 mg) into pouches reduced the release of keratan sulfate from cartilage into serum to within control levels.

Cartilage Studies in a Rabbit Model of Inflammatory Arthritis

A model of arthritis described originally by Page-Thomas (175), in which a single IA injection of a preformed hyaluronan-lysine complex (PC) was injected into rabbit joints, was used to evaluate the ability of CaPPS to preserve cartilage and reduce synovial and serum levels of inflammatory

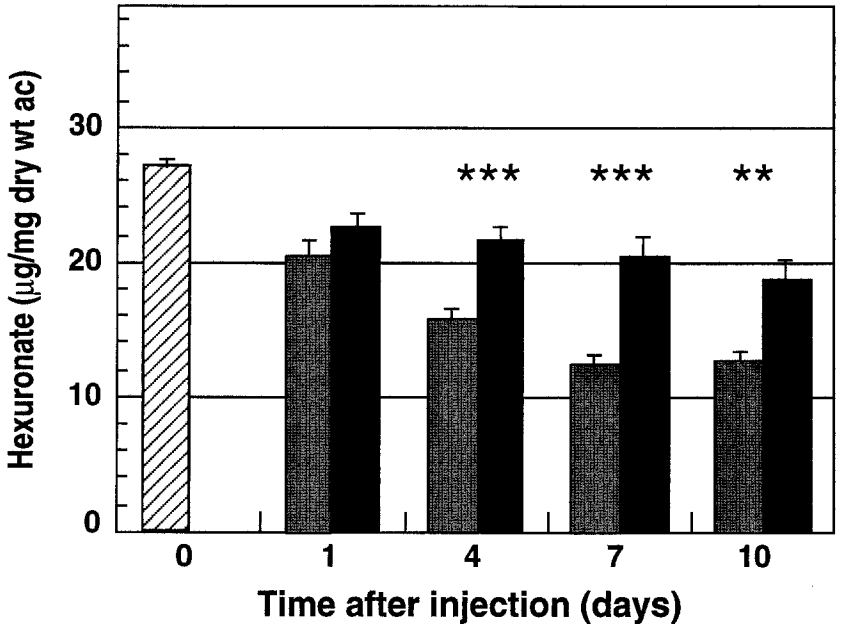

Fig 15. Proteoglycan content (measured as hexuronate) of joint articular cartilage from non–drug-treated PC-injected (arthritic) (▒) and CaPPS-treated (■) (10 mg/kg per os) NZ white rabbits 1, 4, 7, and 10 days after initiating arthritis. Cartilage PG content of non–PC-injected non–drug-treated animals is also shown (▨). Significant differences between values for CaPPS and non–CaPPS-treated arthritic animals were apparent on days 4, 7, and 10 ($P < .005$) (***$P < .0005$).**

mediators (176, 177). In the earlier study (176), CaPPS was given orally at doses of 5, 10, and 75 mg/kg every 48 hours, starting 7 days before PC injection and for 10 days thereafter. In the non–drug-treated group, PC injections elevated serum interleukin-6 (IL-6) activity, synovial fluid white cells, and PGE_2 levels over the first 7 days postinjection. Cartilage PG content, as well as ex situ biosynthesis of PGs, also were depressed over the same period. CaPPS at 10 mg/kg by mouth (PO) decreased serum IL-6 levels on days 1, 7, and 10 but not synovial fluid white cell counts or PGE_2 concentration in the PC-treated joints. Cartilage PG content and biosynthesis were maintained by this dosage of CaPPS, as well as chondrocyte phenotypic expression (176). In a later study (177), CaPPS at 10 mg/kg PO was again shown to maintain not only cartilage PG content (Fig 15) relative to non–drug-treated PC controls but also the in vivo biosynthesis of these moieties, but only on day 7 ($P < .05$) (Fig 16). In this study, IL-6 was elevated in synovial fluid on day 1 of the non–drug-treated PC and saline-injected joints, but these levels were diminished in the corresponding drug-treated group relative to the control group (Fig 17). CaPPS at oral doses of 10 mg/kg and above also were reported to reduce synovial lining proliferation, cellular infiltration, exudation, and angiogenesis in joints of PC-injected animals (178).

Canine Model of OA Induced by Transection of the Anterior Cruciate Ligament (ACL)

Destabilization of canine joints by transection of the anterior cruciate ligament (ACL) has been widely used by numerous investigators to produce an animal model of OA (see review by Pritzker [179]). Although open surgery with careful hemostasis has shown that this model is only slowly progressive, closed transection using a stab incision leads to a greater inflammatory response and more rapid cartilage degradation (180). Rogachefsky et al (152) used a closed ACL transection approach to evaluate the effect of NaPPS alone and in combination with rhIGF-1 on cartilage histological changes, PG content, and levels of MMPs and TIMP. NaPPS was administered at 2 mg/kg intramuscularly for 4 weeks (from week 3 to week 6), whereas rhIGF-1 was given intraarticularly (1 μg; 3 × weekly) on

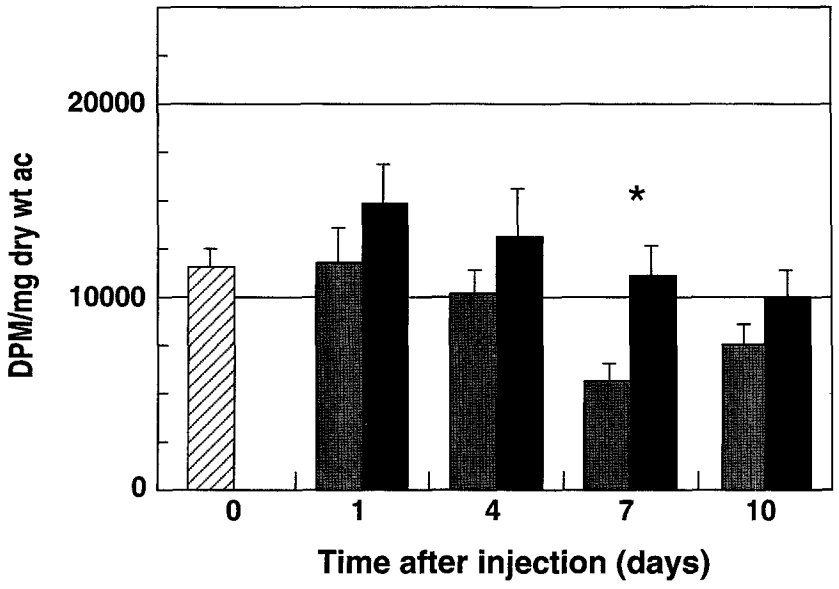

Fig 16. In vivo incorporation of ^{35}S into joint articular cartilage PGs of non–drug-treated PC-injected (arthritic) (▒) and CaPPS-treated (■) (10 mg/kg per os) NZ white rabbits 1, 4, 7, and 10 days after initiating arthritis. ^{35}S-PG levels are also shown for cartilage of non–PC-injected non–drug-treated animals (▨). Although CaPPS stimulated mean uptake of ^{35}S into PGs on days 1 and 4 relative to PC controls, it only showed statistical significance on day 7 (*$P < .05$).

the same occasion. At the termination of the experiment (week 12), Mankin's scores of cartilage injury were lower in the PPS and rhIGF-1/PPS group than the rhIGF-1–treated group and the non–drug-treated OA controls (Fig 18). Although the decrease in cartilage collagen content was not halted by NaPPS alone, the combination with rhIGF-1 restored it to normal levels (Fig 19). In contrast, the PG content of cartilage, relative to non–drug-treated OA controls was maintained by both PPS and the PPS/rhIGF treatments (Fig 19). Active and total MMP levels were significantly lower in cartilage from the NaPPS-treated group than that from the non–drug-treated OA group (Fig

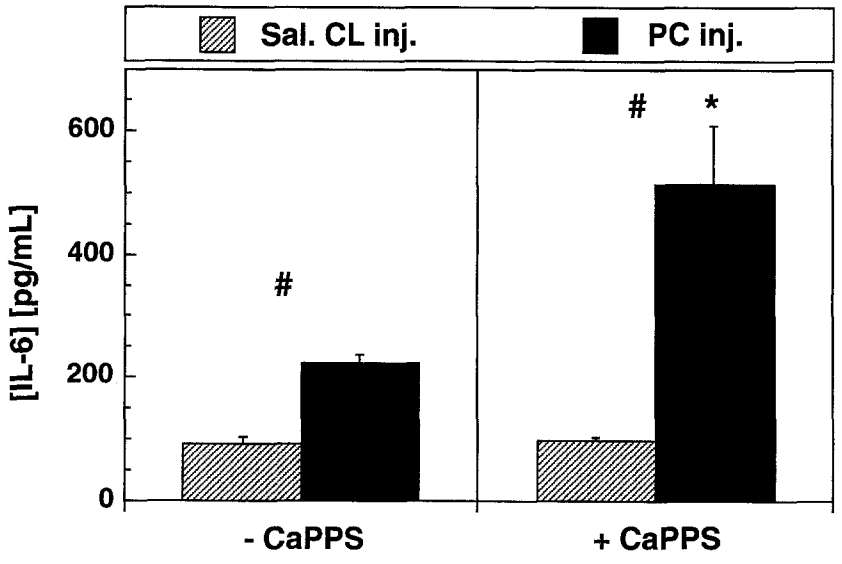

Fig 17. Concentration of interleukin-6 in synovial fluid on day 1 of saline or PC-injected (arthritic) (n = 4) rabbit joints of non–drug-treated (–CaPPS) or animals treated with CaPPS (10 mg/kg per os). Results show mean ± SEM. PC inj. *v* sal. inj. #$P < .05$; no drug *v* CaPPS *$P < .05$.

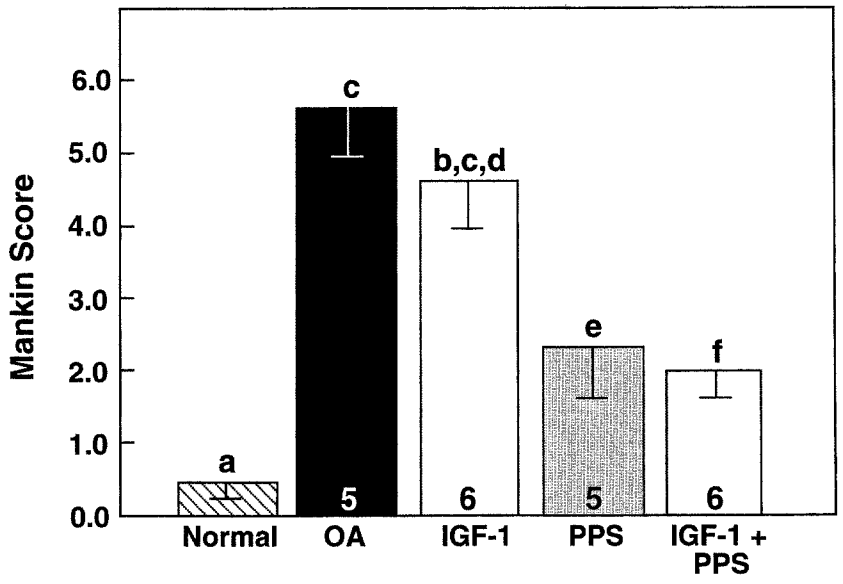

Fig 18. Cartilage scores from the canine OA study assessed using the Mankin system (162). The values shown are the mean ± SEM; the number of samples is shown at the base of each bar (a) normal *v* IGF-1/NaPPS ($P = .018$); (b) IGF-1 *v* IGF-1/NaPPS ($P = .009$); (c) normal *v* IGF-1 ($P < .001$); (d) IGF-1 *v* NaPPS ($P = .035$); (e) NaPPS *v* OA ($P = .007$); (f) IGF-1/NaPPS *v* OA ($P < .001$). Both NaPPS alone, or in combination with IGF-1, reduced cartilage damage in this model. (Data from Rogachefsky et al [152].)

20), whereas levels of TIMP were maintained in the NaPPS and NaPPS/rhIGF-1 treated OA dogs to within normal cartilage values (Fig 21). The preservation of cartilage integrity by NaPPS and the combination of NaPPS and rhIGF-1 in this model were considered by the authors to be primarily mediated by the ability of NaPPS to attenuate the anti-catabolic activities of the MMPs in joint tissues.

SYNOVIAL INFLAMMATION IN OA

Although OA is considered by many to be restricted to pathological events that occur in articular cartilage and bone, synovial inflammation is also a prominent feature of the clinical disease (181-183). It is known that synovial fluid from OA patients contains significant levels of inflammatory mediators and other markers of immune cell activation (184-188). Revell et al (25) examined immunohistologically synovial membrane biopsy specimens from 20 OA patients using monoclonal antibodies. Lymphoid follicles containing T helper and T suppressor lymphocytes, B lymphocytes, and macrophage expressing human leukocyte antigen-DB (HLA-DB) were present in five cases. In half of the OA samples, diffuse cellular infiltrates containing granulocytes, T and B lymphocytes, and HLA-DR–positive macrophages were identified.

The presence of lymphoid follicles and immunocompetent cells in OA synovial tissue were also confirmed in an arthroscopic and immunohistological study undertaken by Lindblad and Hedfors (23). The blood and synovial fluid lymphocyte subsets in rheumatoid arthritis (RA) and OA patients was investigated by Kuryliszyn-Moskal (186). Although peripheral blood analysis from RA patients showed much higher HLA-DR$^+$ and IL2R$^+$T lymphocytes compared with OA blood levels, the cell populations in synovial fluids were not significantly different from each other. Collectively, these studies confirm that synovial inflammation is a prominent feature of OA and is characterized by focal hyperemia, edema, and villous hypertrophy with dilation of venules and arterials arising from the action of the classical inflammogens, which in themselves may contribute to the symptoms of OA.

In a recent study by Smith et al (26), the levels of cytokines (IL-1α and β, TNF-α), as determined using in situ hybridization and biotin-labeled riboprobes, in synovial membranes from OA patients

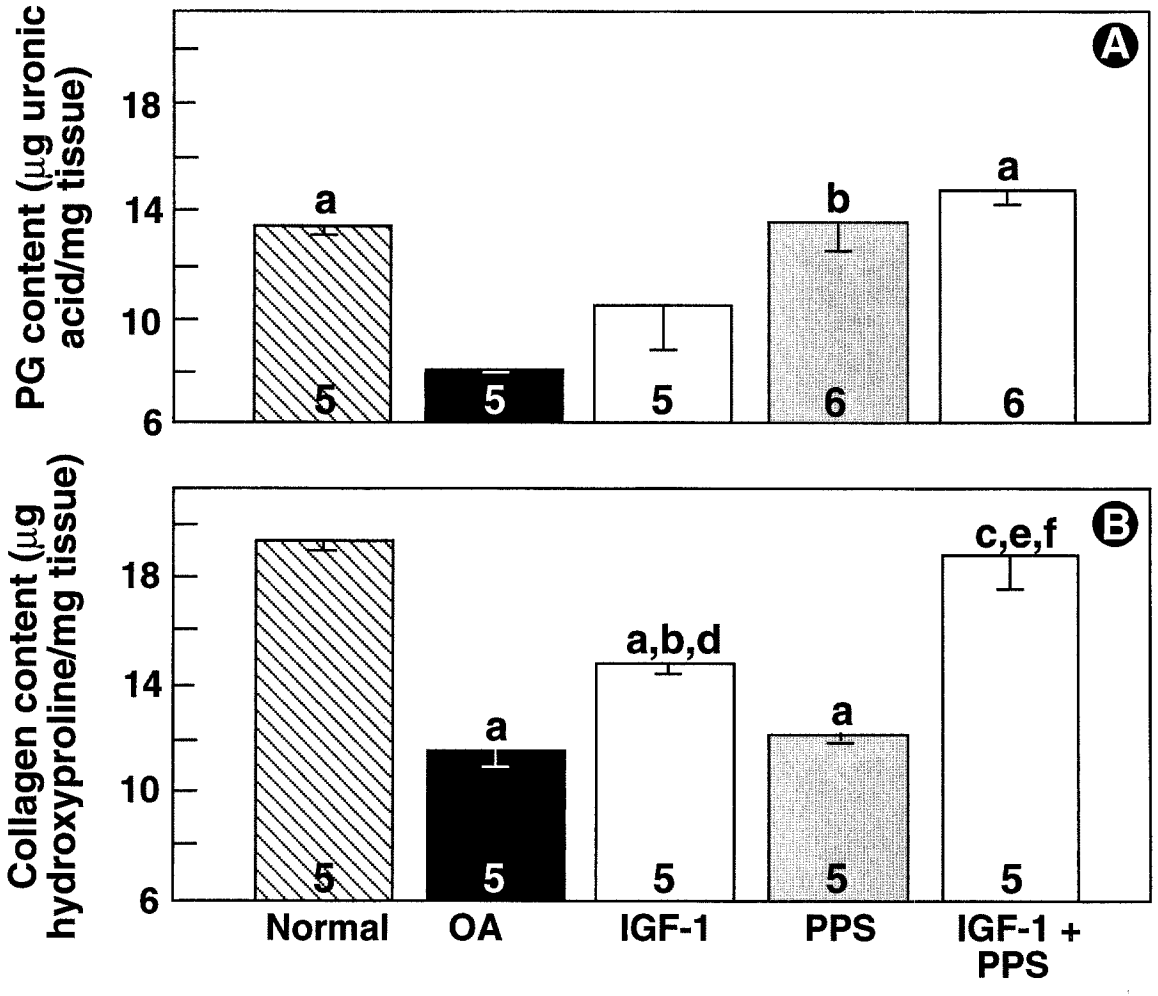

Fig 19. Uronic acid (a measure of PG content) (A) and hydroxyproline (a measure of collagen content) (B) of articular cartilage in the different study groups of the canine OA study. Values are expressed as mean ± SEM in mg/mg wet weight tissue; the number of samples is shown at the base of each bar. Significant differences between groups for uronic acid includes (a) normal *v* OA and IGF-1/NaPPS *v* OA ($P < .001$); (b) NaPPS *v* OA ($P = .002$). Differences between hydroxyproline measurements for groups include (a) OA *v* normal, IGF-1 *v* normal, and NaPPS *v* normal ($P < .001$); (b) IGF-1 *v* OA ($P = .003$); (c) IGF-1/NaPPS *v* OA ($P < .001$); (d) IGF-1 *v* NaPPS ($P = .003$); (e) IGF-1/PPS *v* NaPPS ($P < .005$); (f) IGF-1/NaPPS *v* IGF-1 ($P = .05$). (Modified from Rogachefsky et al [152].)

were reported to be comparable to levels in membrane from joints of RA patients (Fig 22). Furthermore, the activities of these cytokines and the downregulation of the IL-1 receptor antagonist (IL-1ra) correlated directly with grade of disease severity. These data were consistent with the findings of Wagner et al (189), who used polymerase chain reaction methods to quantitate cytokines in OA synovial biopsy specimens, and Roivainen et al (190) and Marok et al (191), who demonstrated oncoproteins and nuclear factor transcription factors for proinflammatory mediators in synovia of OA patients.

The cause of synovial inflammation in OA has not been rigorously established; however, it is likely that it arises secondary to breakdown of articular cartilage. Adult articular cartilage is avascular and thus resides in an immunologically privileged location. After traumatic injury and OA progression, cartilage becomes fibrillated and

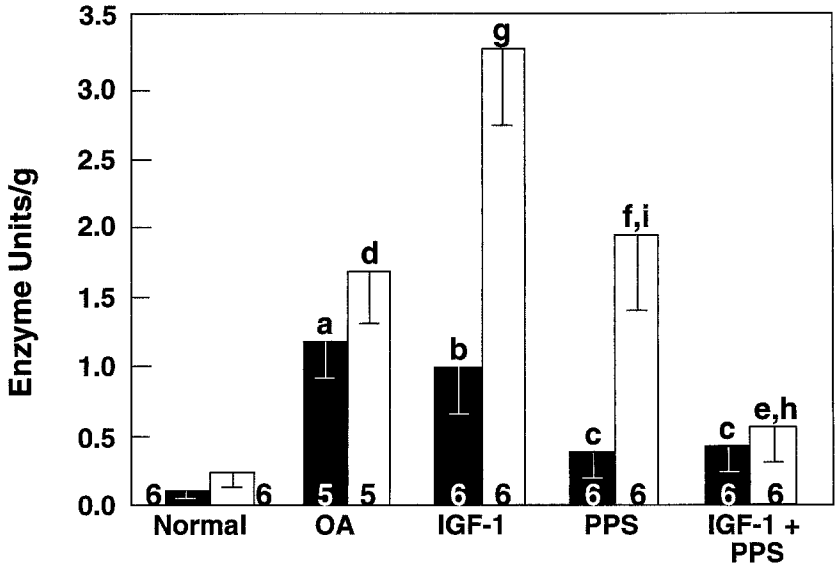

Fig 20. Levels of active (■) and total (active + latent) (□) matrix metalloproteinases (MMPs) in articular cartilage from the various groups of the canine OA study. The values are the mean ± SEM enzyme units/g cartilage; the number of samples is shown at the base of each bar. (a) OA *v* normal ($P = .002$); (b) IGF-1 *v* normal ($P = .02$); (c) NaPPS *v* OA and IGF-1/NaPPS *v* OA ($P = .03$); (f) NaPPS *v* normal ($P = .01$); (g) IGF-1 *v* normal ($P < .001$); (h) IGF-1/PPS *v* IGF-1 ($P = .002$); (i) NaPPS *v* IGF-1/NaPPS ($P = .04$). (Modified from Rogachefsky et al [152].) The combination of IGF-1 and NaPPS effectively normalized cartilage active and latent MMP levels. However, IGF-1 alone exacerbated levels of total MMPs.

eroded, and matrix fragments are released into synovial fluid (192-196). These cartilage-derived products are antigenic (197-202) and have been shown to elicit synovial inflammation when injected into rabbit joints (197, 201, 203-207). The strong antigenic nature of components of the cartilage matrix also has been shown in vitro by the activation of macrophages with PG fragments (208), type II collagen (198, 199, 209) other collagens (199, 202) and chondrocyte membrane proteins (200). It is clear, therefore, that the physical fragmentation and proteolytic degradation of cartilage in OA joints generate a host of immunogens that, when released into synovial fluid, are capable of activating resident synovial macrophage and establishing synovitis.

ANTI-INFLAMMATORY ACTIVITIES OF CaPPS AND NaPPS

The potent antiinflammatory effects of NaPPS were demonstrated initially by Kalbhen and coworkers (210-216), using edemas induced in rat paws by injection of a variety of agents including dextran, formaldehyde, trypsin, hyaluronidase, carrageenan, or kaolin. In all experiments, a dose-dependent antiedema response was observed using subcutaneous administered NaPPS over the concentration range of 25 to 100 mg/kg (Table 4). The optimal time for administration of the drug was found to be 60 minutes before edema initiation (Fig 23). From these studies, Kalbhen concluded that, in contrast to sodium salicylate, phenylbutazone, or indomethacin, NaPPS was effective against a wide range of inflammogens, possibly because of stabilization of the peripheral vascular system and improvement of the microcirculation in the inflamed tissues stimulated by the drug.

Studies by Walb, Loos, and Hadding (217) showed that NaPPS possesses marked anti-complementary activities. NaPPS was 10 times more potent than HEP in preventing the lysis of sensitized erythrocytes by a complement preparation over the concentration range 5.0 to 8.3 μg/mL. In vitro NaPPS also directly inhibited the complement C1-esterase, the 50% effective dose (ED50) being between 7 and 8 μg/mL.

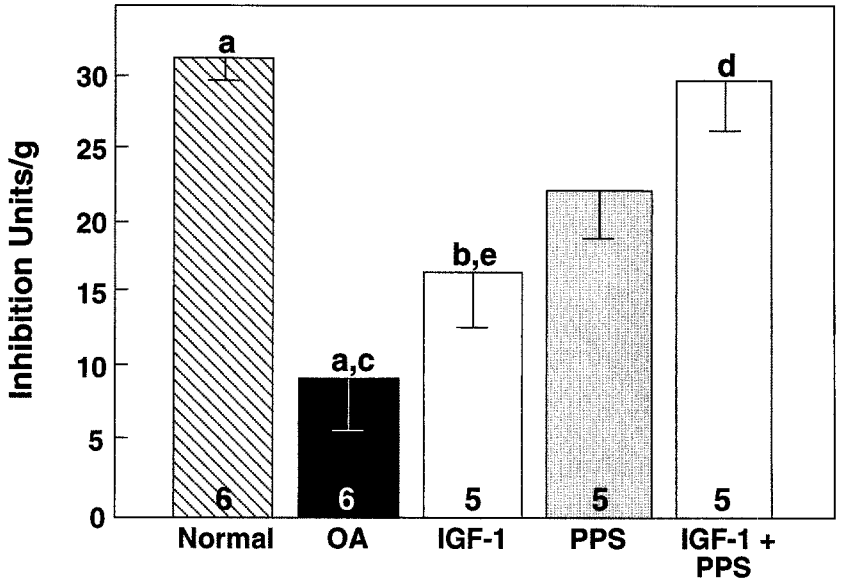

Fig 21. Levels of tissue inhibitor of metalloproteinases (TIMP) in articular cartilage from the various groups of the canine OA study. The values are the mean ± SEM inhibitor units/g cartilage; the number of samples is shown at the base of each bar. (a) OA *v* normal ($P < .001$); (b) IGF-1 *v* normal ($P = .007$); (c) normal and OA *v* NaPPS ($P = .04$); (d) IGF-1/NaPPS *v* OA ($P < .005$); (e) IGF-1 *v* IGF-1/NaPPS ($P = .03$). (Modified from Rogachefsky et al [152].) NaPPS alone and IGF-1 alone and in combination with NaPPS showed improvement in OA cartilage TIMP levels.

Berthoux et al (218-220) confirmed the anti-complementary activity of NaPPS both in vitro and in vivo. In vitro studies were undertaken with human serum, where the drug was found to be effective in rapidly reducing total hemolytic complement activity. Anti-complement activity was shown in patients using an intravenous dosage of 100 mg NaPPS every 8 hours, that is, 300 mg over 24 hours. In patients with established hypocomplementemia, NaPPS was not effective; however, in patients with normal complement levels or moderate hypercomplementemia, serum total hemolytic complement activity (CH50) concentration could be markedly reduced by use of the drug. The dose-response curve for this activity was sigmoidal, with an ED50 of approximately 0.08 mg/mL in normal human serum. PPS, however, failed to produce a significant change in serum levels of C4 or properdin factor B, as determined by radial immunodiffusion techniques. Conversely, C2, C3, and C4 were totally inhibited, and C1 and C5 were partially inhibited by NaPPS. These studies led Berthoux et al (218-220) to suggest that the in vivo anti-complement activities of NaPPS were sufficiently strong to decrease the release of humoral mediators of inflammation when the drug was used at a dose of 100 mg intramuscularly. This suggestion was recently confirmed by Kilgore et al (221), who showed that NaPPS inhibited complement-mediated erythrocyte lysis in vitro and reduced myocardial tissue injury during activation of the complement cascade.

Using a new model of inflammatory arthritis developed in Wistar rats by the injection of the arthritogen, *Mycobacterium tuberculosis* (Mtb) (1 mg) in squalane (0.1 mL) into the tail followed by another injection of Mtb (0.5 mg in 10 mL phosphate-buffered saline [PBS] with 0.05% Tween-20) into preformed subcutaneous air pouches, it was shown that daily subcutaneous doses of CaPPS given at 10 and 20 mg/kg reduced tail and paw swelling as well as the production of inflammatory mediators in pouch fluids (PF) (Figs 24-27) (222).

PF was collected at killing on day 18, and the PF volume, total and differential white cell count, nitric oxide (NO·) (as nitrite ion), a known mediator of cartilage destruction in OA (37, 223, 224), HY, and PGE_2 were determined using published methods. The PF and serum levels of IL-6 also

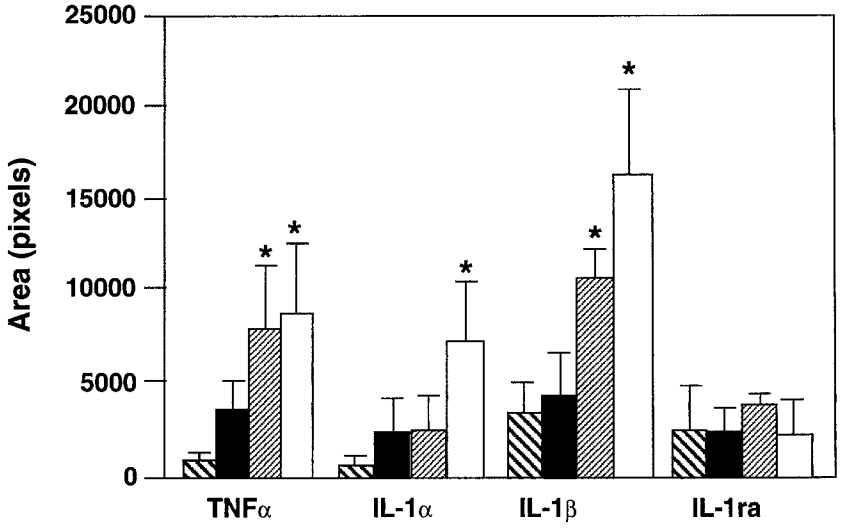

Fig 22. Quantitation (by computer-assisted image analysis) of immunohistochemical labeling for various cytokines and IL-1 receptor antagonist (IL-1ra) present in biopsy specimens of synovial lining from joints of normal subjects and subjects with various grades of OA as determined radiographically. (▨ = normal, ■ = early; ▧ = moderate; ▩ = severe OA). (Modified from Smith et al [26].) *Different from normals ($P < .05$). Note that cytokines increased with increase in OA grade severity but IL-1ra did not.

Table 4: Anti-edema Effects of Subcutaneously Administered NaPPS on Experimentally Induced Edema in the Rat Paw With Various Agents

Agent/Dose	No. of Animals (n)	NaPPS Dosage (mg/kg)	% Swelling	% Inhibition of Edema	Significance Between Doses
Trypsin (0.05 mg in 0.1 mL)	10	0	59.1 ± 1.9	0	—
	10	25	45.4 ± 1.8	23.1	$P < .001$
	10	100	29.7 ± 1.9	49.5	$P < .001$
Hyaluronidase (50 IU = 0.1 mg in 0.1 mL)	10	0	26.9 ± 1.0	0	—
	10	25	25.0 ± 0.7	7.1	NS
	10	100	13.0 ± 0.8	53.8	$P < .001$
Dextran (6 mg in 0.1 mL)	10	0	87.0 ± 2.3	0	—
	10	25	73.7 ± 2.2	15.3	$P < .001$
	10	100	48.9 ± 3.0	44.0	$P < .001$
Carragenin (0.2 mg in 0.1 mL)	10	0	42.1 ± 1.9	0	—
	10	50	16.1 ± 0.5	61.8	$P < .001$
	10	100	17.6 ± 0.6	58.2	$P < .001$
Formalin (3%:0.1 mL)	10	0	33.6 ± 1.0	0	—
	10	50	34.0 ± 1.2	4.5	NS
	10	100	29.3 ± 1.0	17.7	$P < .001$

NOTE. $P < .05$ significant.
Abbreviation: NS, nonsignificant.
Data from Kalbhen et al (211, 213, 214, 216).

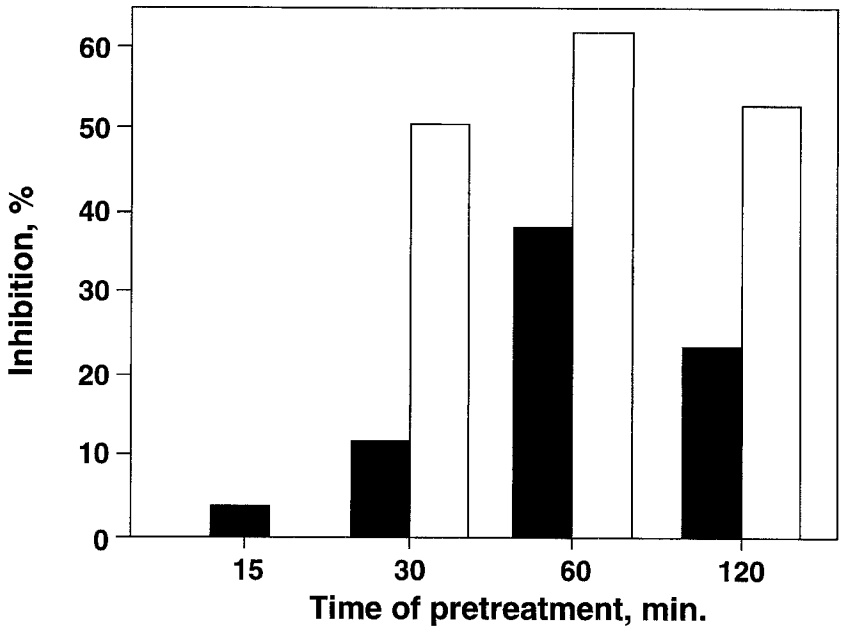

Fig 23. Inhibitory effect of NaPPS (50 mg/kg) on hyaluronidase induced edema (■) and carrageen-induced edema (▨) as a function of time of pretreatment with the drug via the subcutaneous route. (See also Table 4.) Note that peak anti-inflammatory effects of NaPPS occurred approximately 1 hour after drug administration, a result consistent with its rate of distribution.

were determined by using a bioassay (proliferation of 7TD1 cells).

Although CaPPS had minimal effects on PF volume or PGE_2 levels at 20 mg/kg, it reduced total white cells (Fig 26), NO· as determined by nitrite ion concentration (Fig 27), and HY concentration (Fig 28). Mean IL-6 levels in PF were elevated by CaPPS at 5 mg/kg but reduced at 20 mg/kg, although these changes were not significant (Fig 29A). However, IL-6 activity was depressed in serum, being significant at CaPPS doses of 5, 10, and 20 mg/kg (Fig 29B). These studies confirmed the antiinflammatory and immunomodulatory activities of CaPPS and were consistent with the earlier studies of Kalbhen et al (210-216) using NaPPS.

In an earlier study by Bansal et al (225), in which a peptone-stimulated subcutaneous rat air-pouch model was used, anti-cytokine activities of CaPPS when given subcutaneously over the dosage range of 0.5 to 10 mg/kg also were demonstrated. In this model, CaPPS showed no effect on volume, influx of white cells, or PGE_2 in PFs. However, a 38% reduction in TNF-α activity was obtained when dosages of 2.5 to 10 mg/kg were used. In this dose range, IL-6 activity in PFs also were increased (21% to 47%), whereas the levels in sera declined 40%. PF levels of IL-1β were not affected by administration of the drug. The net outcome of changing the respective levels of IL-6 and TNF-α but not IL-1 in inflammatory fluids in this animal model is difficult to interpret. However, elevated TNF-α levels have been detected in synovial fluid from human arthritic joints (226) and in synovial fluid from an experimental model of OA in dogs (227). IL-1 and TNF-α have been shown to induce the synthesis of IL-6 in connective tissue cells and chondrocytes (228), suggesting that IL-6 may act as an antiinflammatory cytokine that counteracts the effects of IL-1/TNF-α (229-231). It also suppresses IL-1 and TNF production by stimulated blood mononuclear cells (232), increases the production of PGs and collagen by human fibroblasts, and induces chondrocyte proliferation and restoration of normal chondrocyte phenotype in tissue culture (231). Furthermore, IA administration of IL-6– to IL-6–deficient (knockout) mice reduced cartilage destruction when they were used as a model of arthritis (230). There is also evidence that IL-6 increases the production of TIMP by chondrocytes and synovial fibroblasts (227, 231, 233). Anti-arthritic drugs such as dexamethasone, cyclophosphamide, and cyclosporin A normalized IL-6 levels in sera of rats with adjuvant arthritis, whereas indomethacin did not (234).

Collectively, these reports suggest that CaPPS, by increasing IL-6 levels in joints, antagonizes IL-1– and TNF-induced catabolic activities by chondrocytes, thereby maintaining articular carti-

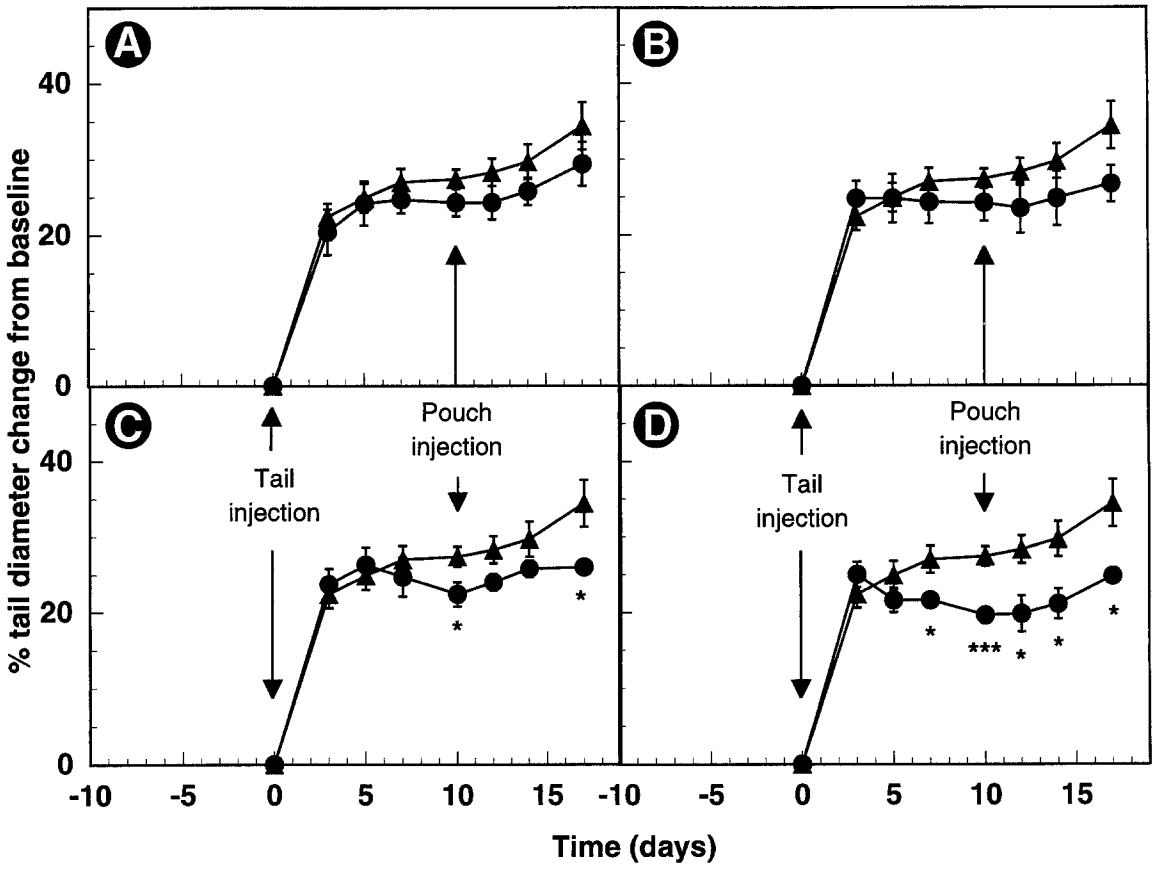

Fig 24. Percentage tail diameter change over 17 days of Mtb-treated rats injected subcutaneously with no CaPPS (▲) or CaPPS (●) at (A) 2.5, (B) 5, (C) 10, and (D) 20 mg/kg daily. Significant differences between treated and nontreated groups. (*$P < .05$; **$P < .005$; ***$P < .0005$) only occurred with the doses of 10 and 20 mg/kg CaPPS.

lage homeostasis. This process also would be supported by the ability of IL-6 to increase TIMP production by chondrocytes.

EFFECTS OF NaPPS AND CaPPS ON SYNOVIAL FIBROBLAST METABOLISM AND SYNTHESIS OF HYALURONAN

HY, the major nonproteinaceous component of joint synovial fluid, confers the unique rheological properties, which include exceptionally efficient lubrication of articular cartilage and periarticular tissues (29, 72, 235-242) to this medium. In rheumatoid and osteoarthritic joints, synovial fluid HY concentration and molecular weight are decreased (243). Because the rheological effects of HY are dependent on its molecular weight and concentration (29, 235-242), a decline in either or both of these parameters reduces the ability of synovial fluid to efficiently lubricate and protect articulating surfaces. Furthermore, recent research has shown that molecular interactions occur between HY and inflammatory mediators (including platelet-activating factor [PAF] and phospholipids) and cell surface receptors in a molecular weight–dependent manner, which could account for the improvement in symptoms after IA injection of high-molecular-weight HY into OA joints (244-246). Of particular significance has been the identification of the cell surface receptor for HY as CD44. This receptor is present on a variety of cells, including peripheral blood monocytes and macrophages (247-249), synovial fibroblasts (250), and chondrocytes (251), all of which are exposed to HY in the OA joint. Diminished availability and a reduction in molecular weight of synovial fluid HY therefore could have profound effects on not only

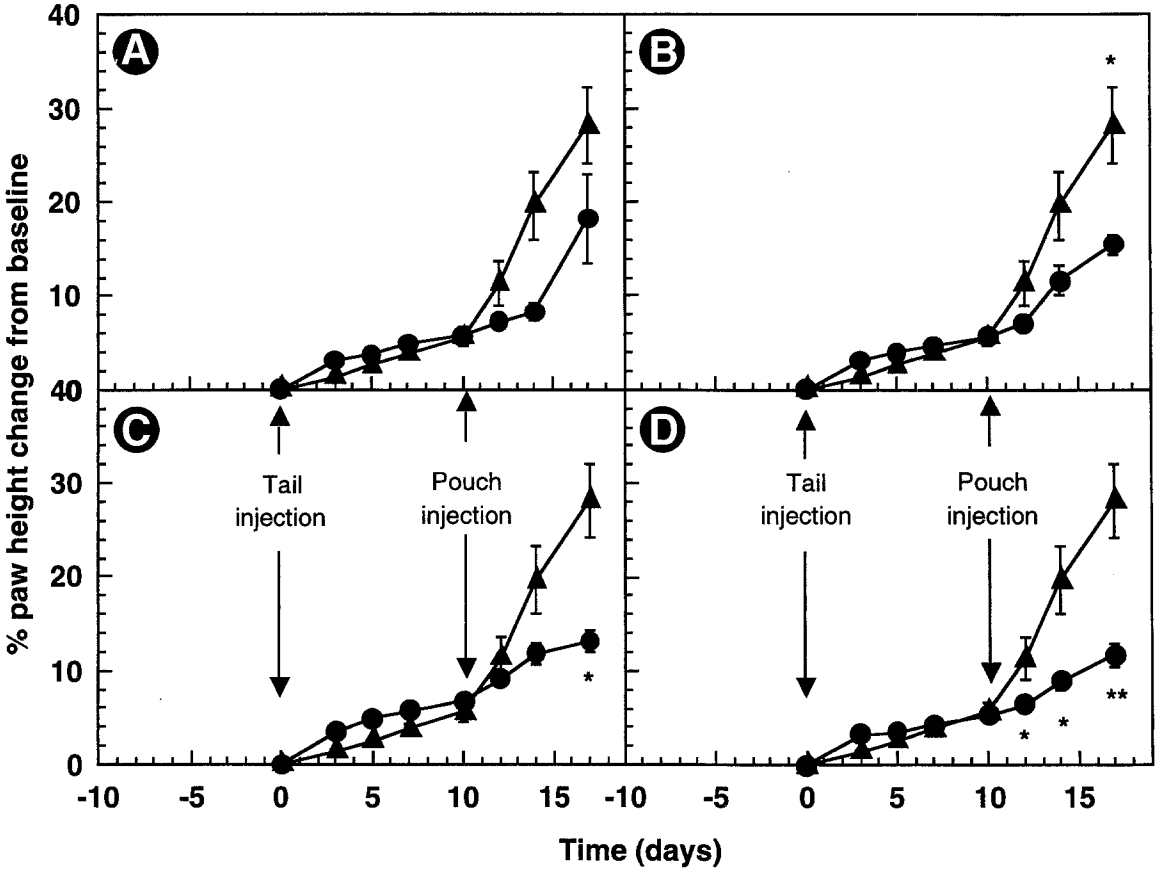

Fig 25. Percentage paw swelling changes over 17 days of Mtb-treated rats injected subcutaneously with no CaPPS (▲) or CaPPS (●) at (A) 2.5, (B) 5, (C) 10, and (D) 20 mg/kg as per protocol. Significant difference between treated and nontreated groups (*$P < .05$; **$P < .005$) only occurred with doses of 10 and 20 mg/kg CaPPS. (Modified from Smith et al [222].)

the rheological properties of synovial fluid but also on synovial and inflammatory cell functions and their macromolecular expression.

Synovial fluid HY is almost exclusively synthesized by type B cells of the synovial lining (252-255). These fibroblasts (synoviocytes), when isolated from synovial joints and established in culture, retain their phenotypic expression and elaborate HY into culture media (254, 256). Fibroblast cultures derived from patients with RA or OA show aberrant biosynthesis of HY, reflecting the abnormal metabolic status of the joints from which they were obtained (254, 256). Generally, the amount of HY synthesized is less than that from normal synoviocytes, and its molecular weight is reduced.

Cultures of human synovial fibroblasts derived from RA and OA patients showed a concentration-dependent stimulation of HY synthesis when exposed to NaPPS and CaPPS (116, 257). In both cell lines, maximum stimulation occurred at drug concentrations of 0.25 μg/mL. In the cells from RA joints at concentrations in excess of 1.0 μg/mL HY synthesis was inhibited. However, inhibition was not observed in the OA cell line when concentrations up to 2.0 μg/mL were used (Fig 30).

An in vivo stimulatory effect on HY synthesis was found in fluids of the rat air-pouch model treated with NaPPS (258). As already indicated, the lining of the pouch consists of fibroblasts with many similarities to synovial synoviocytes (169-171). In these experiments, NaPPS or GAGPS at 10 mg/kg was injected daily for 7 days into peptone-inflamed air-pouches in which rabbit cartilage had been implanted. Control rats received an equal

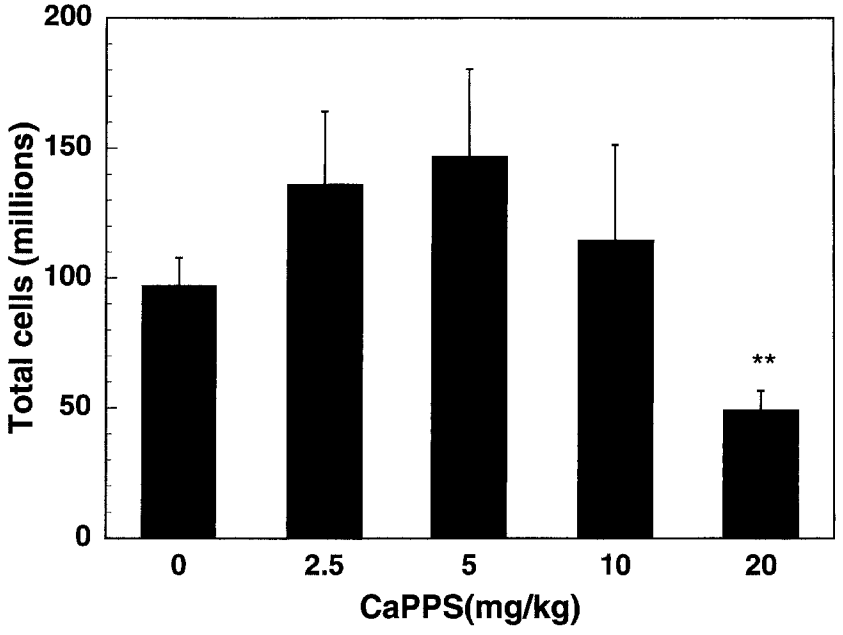

Fig 26. Effect of increasing CaPPS dosages on the total white cell numbers in the airpouch fluid of Mtb-treated rats 17 days after Mtb tail injection (see text for method). Although cell levels were elevated in pouch fluid at low doses (<10 mg/kg), a significant depression occurred at 20 mg/kg CaPPS ($P < .005$).**

volume of sterile saline for 7 days into identically prepared rat air-pouches. PFs were analyzed specifically for HY content, using an enzyme-linked immunoabsorbent-inhibition assay (ELISIA), where it was shown that the concentration of HY accumulating in PFs injected with NaPPS was significantly higher than in pouches receiving saline or GAGPS (Fig 31). Moreover, the molecular weight of the HY produced by pouch fibroblasts in the presence of PPS was also increased, as shown by gel-filtration chromatography (258).

This finding was confirmed in human studies, where NaPPS was administered intraarticularly into joints of patients with RA (259) or OA (260). In the RA study, significant increases in the molecular weight of synovial fluid HY was obtained after 3 to 6 intra-injections of 50 mg NaPPS (Fig 32).

In the OA studies, 28 patients with a clinical and

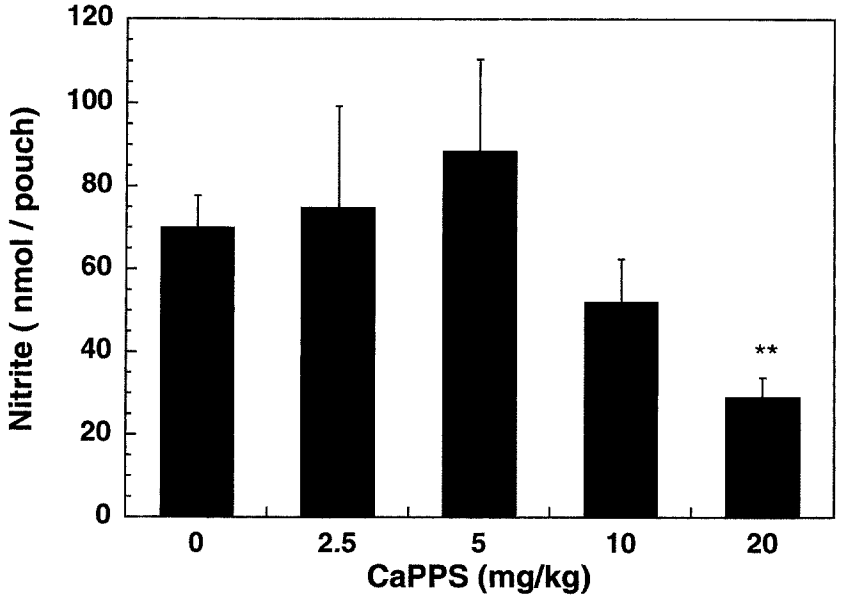

Fig 27. Effect of increasing CaPPS dosages on the total nitrite (a metabolite of NO·) concentration in the airpouch fluid of Mtb-treated rats 17 days after Mtb tail injection (see text for method). Note the similarity to white cell profile (Fig 26), suggesting that the origin of NO· was from infiltrating cells ($P < .005$).**

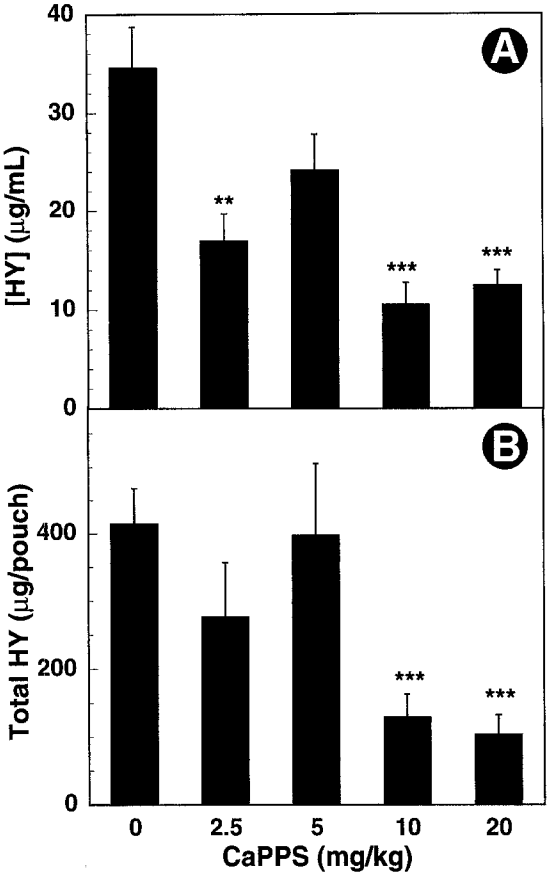

Fig 28. Effect of increasing CaPPS dosages on (A) concentration and (B) total amount of hyaluronan (HY) in the airpouch fluid of Mtb-treated rats 17 days after Mtb tail injection (see text for method) ($P < .005$; ***$P < .0005$). Significantly, the levels of HY were reduced in pouch fluid at 10 and 20 mg/kg CaPPS doses, which also were found to afford an anti-inflammatory effect (see Figs 24 and 25).**

radiological diagnosis of OA of the knee were evaluated. The treatment regimen consisted of four consecutive weekly IA injections of NaPPS (50 mg) or an equivalent volume of placebo (PBS) given under double-blind conditions. Synovial fluid was sampled before entry and weekly thereafter, centrifuged to remove cells, and the supernatant analyzed using micro-Fourier rheometry to measure stiffness (G′ corr) and viscosity (η') and HY molecular weight by multi-angle laser-light scattering after gel permeation chromatography (GPC/MALLS). For comparative purposes, G′ and η' were combined into a single value to afford the rheological index. After rheological examination, the samples were diluted with buffer (1:5) and applied to the GPC/MALLS system. The weight average molecular weight (M_w) of the purified HY was measured on-line by laser-light scattering after gel chromatography. The results of this study showed that the rheological index of synovial fluid viscosity in the NaPPS-treated group improved by an average of 106% (±66%) of baseline, whereas fluids from the placebo group increased by 9% (±9%). The placebo group values were signifi-

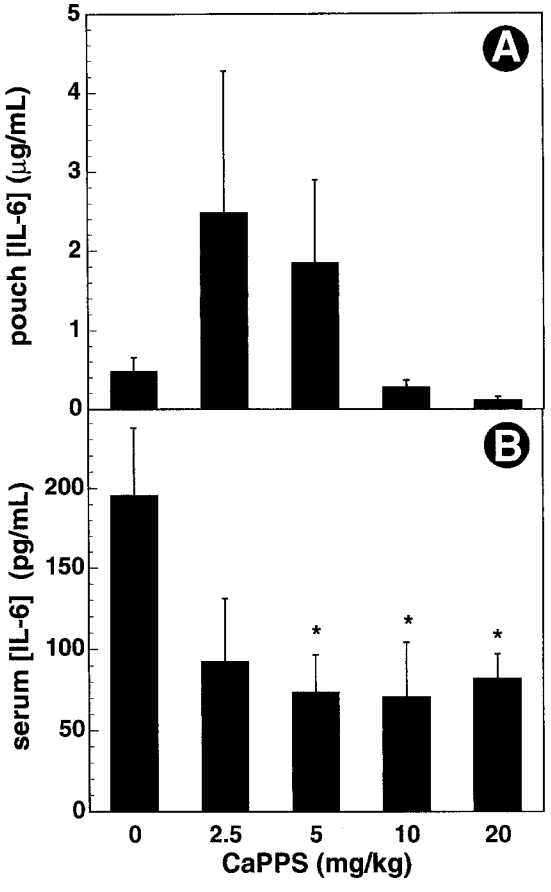

Fig 29. Effect of increasing CaPPS dosages on IL-6 activity in (A) airpouch fluids and (B) serum of Mtb-treated rats 17 days after Mtb tail injection (see text for method). Note that CaPPS increased IL-6 in pouch fluid but decreased it in serum, suggesting different effects of CaPPS on pouch lining cells and circulating leukocytes (*$P < .05$).

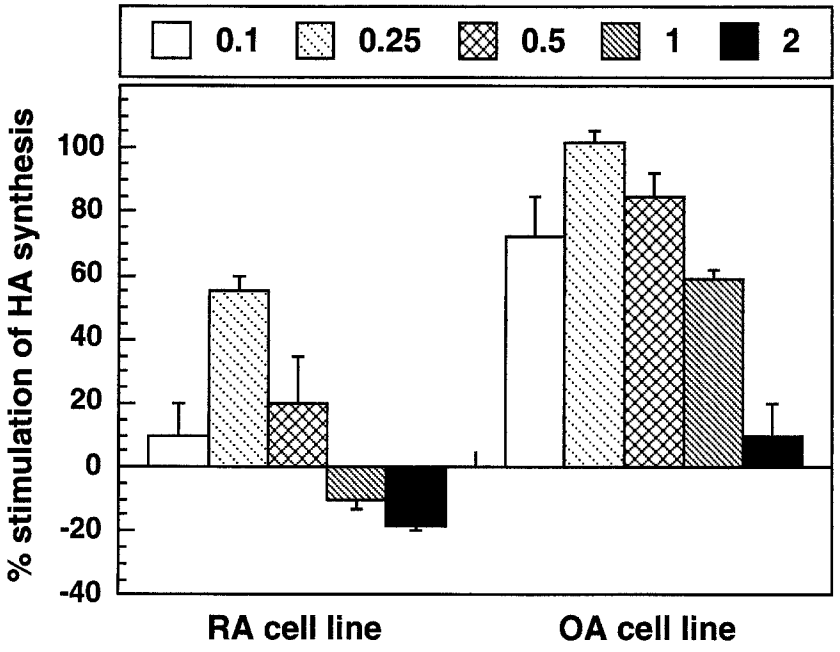

Fig 30. Concentration-determined effect of NaPPS on the synthesis of ^{3}H-hyaluronic acid (hyaluronan) (HA) by synovial fibroblasts derived from rheumatoid arthritis (RA) and osteoarthritis (OA) joints. The cultures were incubated with the various concentrations (μg/mL) of the drug as indicated. (Modified by permission of the publisher from Hutadilok et al, Current Therapeutic Research, 44:849, Copyright 1988 by Excerpta Medica Inc. [257].)

cantly different from those of the PPS-treated group ($P = .05$) (260).

Analysis of the accompanying HY molecular weight changes showed significant differences between the two experimental groups ($P = .016$). HY in fluids from PPS-treated OA patients showed an average M_w increase of 83% (±38%), whereas the corresponding placebo group HY decreased by 23% (±12%) (260).

A study by Ghosh and Hutadilok (261) showed that PPS bound to synovial fibroblasts and was internalized. It was postulated that this process could favorably influence the metabolic functions of these cells and their response to inflammatory mediators. This suggestion was evaluated using the same rabbit model of inflammatory arthritis that was employed to examine the effects of CaPPS on cartilage metabolism (262).

In this study, CaPPS was again given orally at 10 mg/kg every 48 hours over the 10-day experimental period. Rabbits were killed, and synovial fibroblast cultures (RSF) were established from saline-injected and PC-injected joints of drug- and non–drug-treated animals. After three cell passages,

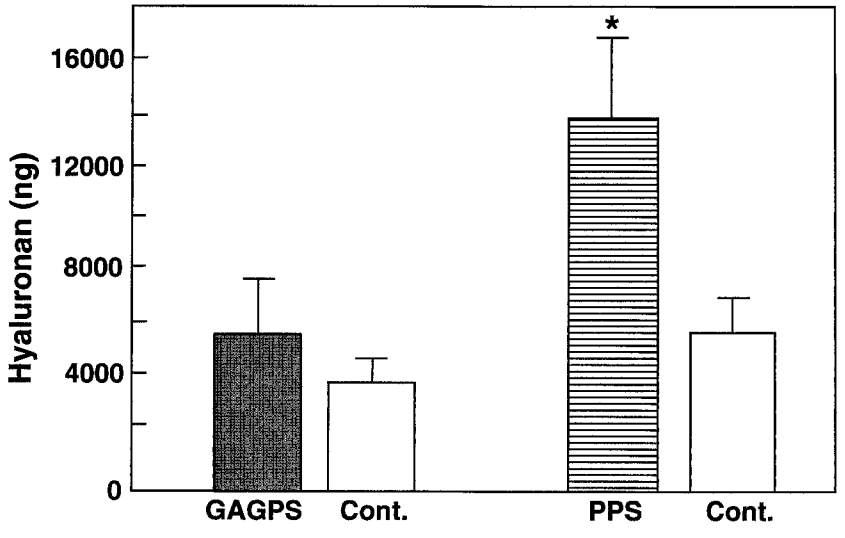

Fig 31. Hyaluronan (HY) levels (as determined by an ELISIA assay; Kongtawelert and Ghosh [362]) in inflamed rat subcutaneous airpouches after daily treatment with NaPPS (PPS) (10 mg/kg) or glycosaminoglycan polysulfate (GAGPS) (10 mg/kg), or without drugs (control). Only NaPPS stimulated HY levels relative to control (*$P < .05$). (Modified from Rheumatology International, Francis et al 1993, 13:62 [258].)

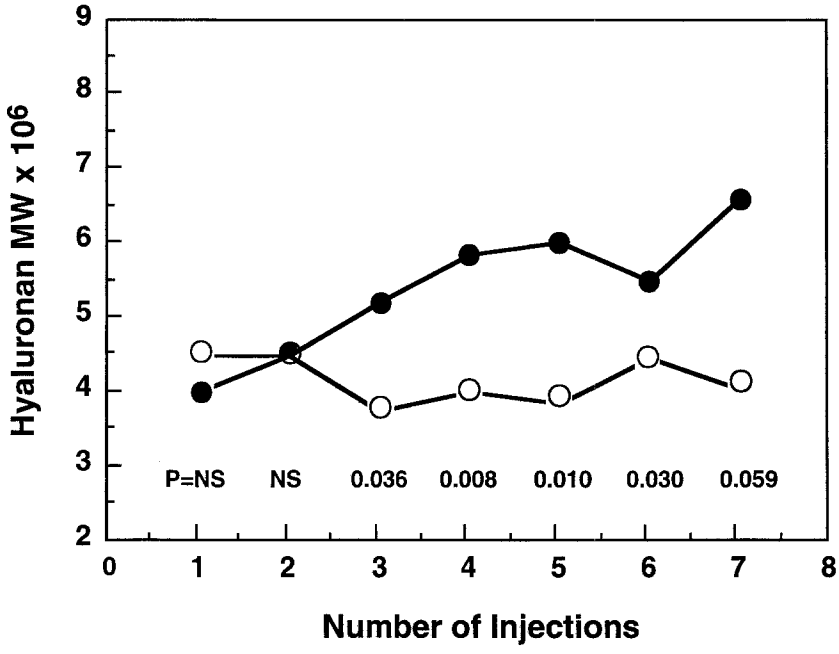

Fig 32. Progressive stimulation of increasing molecular weight of hyaluronan in synovial fluid from knee joints of rheumatoid patients after three or more intra-articular injection of NaPPS (50 mg) (●) compared with injections with saline (○). The *P*-values on the x-axis show that significant difference occurred between NaPPS and saline after three injections of drug. (See ref 259 for details.)

IL-1β (0, 20, 40 pg/mL) was included in the cultures for 24 hours, then DNA, IL-6, and NO· (as the metabolite NO_2) were assayed in the media. In RSF cultures from non–PC-injected joints, IL-1β over the concentration range 20 to 40 pg/mL progressively decreased cell numbers as measured by DNA content ($P < .05$). This antimitotic effect of IL-1β was abrogated in RSF cultures derived from joints of PC-injected animals given oral CaPPS (Fig 33). Production of NO· was stimulated up to 80% by exposure of RSF from PC joints to 40 pg/mL of IL-1 ($P < .01$), but cultures from joints of animals administered oral CaPPS showed reduced NO· production by the RSFs to near basal levels (Fig 33). IL-1β at 20 pg/mL markedly increased IL-6 activity in media of PC-RSF ($P < .005$), but the levels of these cytokines were not reduced by administration of CaPPS (262).

These data are consistent with the hypothesis that CaPPS is capable of modulating the antimitotic effects of IL-1 and its release of NO· from synovial fibroblasts. Although the mechanism(s) responsible for cytoprotection of synovial fibroblasts are unknown, other studies have shown that NaPPS strongly binds to surface receptors of mesenchymal cells followed by internalization of the drug by active receptor uptake and localization into the nucleus (157). It is possible, therefore, that CaPPS may be acting at the gene promoter level of synovial fibroblasts, as described for chondrocytes (156) and smooth muscle cells (157).

THE INTRAOSSEOUS (SUBCHONDRAL) AND SYNOVIAL CIRCULATION IN OSTEOARTHRITIC JOINTS

The need for blood to clot is an essential requirement for survival after trauma, but this process also can occur for reasons unrelated to direct vascular injury. The thrombi that may become localized in capillaries through such an event are normally degraded by proteinases of the fibrinolytic system, the activities of which are carefully regulated by endogenous inhibitors such as plasminogen activator inhibitors (PAI-1, PAI-2) and antiplasmin. Should the balance between the fibrinogenic and fibrinolytic systems become disturbed in favor of the former, then the thrombi formed may localize in capillaries and impede blood flow to the tissue and thus nutrition to its resident cells.

In contrast to articular chondrocytes, which depend on diffusion of nutrients from synovial fluid, osteocytes in subchondral bone are nourished by a well-developed capillary plexus. Osteocyte viability and ability to respond to changing endocrine and mechanical demands is therefore critically dependent on the patency of the vessels that serve these cells. Impaired blood flow due to venous congestion of the intraosseous vasculature

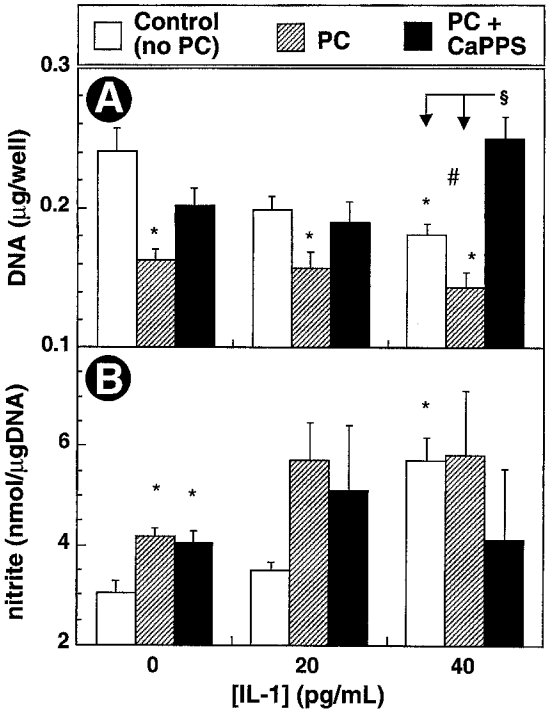

Fig 33. In vitro effects of interleukin-1β (IL-1β) on (A) DNA and (B) NO· radical (measured as nitrite) synthesis by rabbit synovial fibroblasts (RSF) derived from saline (□) and PC-injected (arthritic) joints of non–drug-treated (▨) or arthritic and CaPPS (10 mg/kg PO) treated (■) animals. Results are shown as means ± SEM (n = 3 RSF cell lines). In this study, oral CaPPS provided some cytoprotective effects to RSF with respect to the effects of adding IL-1β to cultures. (*$P < .05$ from saline, #$P < .05$ from saline at same IL-1 concentration, §$P < .05$ from both saline and PC-treated). (Modified from Smith et al [262]).

of OA femoral heads was established more than 40 years ago by microangiography (263) and phlebography (264, 265). These hip studies were complemented by similar findings for the patella-femoral (266, 267) and tibial-femoral (264) joints, as well as in animal experiments (268, 269), where OA-like changes were produced in joints by surgically induced vascular congestion in subchondral bone. The venous hypertension arising in human OA joints as a consequence of a competent arterial inflow coupled with increased resistance to venous outflow may be considerable. In OA femoral heads, the average intramedullary pressure may be greater than 45 Torr, compared with 19 Torr in normal femoral heads (16, 18, 270, 271).

The aforementioned vascular and cellular events can lead with time and joint loading to extensive epiphyseal remodeling. This is seen morphologically as side-by-side resorption and formation of new bone (sclerosis) accompanied by vascular invasion of calcified cartilage, osteophytosis, and fibrillation of articular cartilage (9-12).

Histological examination of OA femoral heads has confirmed the presence of fibrin thrombi and lipid emboli in the microvasculature of subchondral bone (14, 272). Sixteen of 25 femoral heads removed from patients with OA showed widespread loss of osteocyte viability and bone death, as determined histochemically (17). Osteocyte necrosis was longstanding and occurred before the appearance of clinical symptoms (17). Given that the vascular supply to hip and knee joints may be readily compromised (15, 268, 273), it is not surprising that even limited thrombosis could have marked effects on cell metabolism. Because the presence of these thrombi is determined by the local balance between the multiple pathways responsible for clot formation and its dissolution, it could be hypothesized that there exists an intrinsic disturbance in this balance in patients with OA. Indeed, there is evidence to support this view (14, 15, 274).

Hypercoagulability, hypofibrinolysis, and elevated lipids have been found in the plasma of patients with advanced hip OA relative to the same parameters in age-matched non-OA controls (14, 274). Platelet sensitivity to adenosine diphosphate (ADP) was increased, as was factor VIIIc, cholesterol, and triglyceride levels. Significantly, euglobin clot lysis times (ECLT) were prolonged, identifying the patients' innate difficulty in degrading cross-linked fibrin (274). In simple terms, the ECLT may be considered an indicator of the balance between an individual's ability to generate tissue plasminogen activator (t-PA, the serine proteinase required to convert plasminogen to plasmin, Fig 34) and its endogenous inhibitor (PAI-1) (Fig 34). Moreover, raised plasma D-dimer levels in the plasma of OA patients (274) suggests that the fibrin, once deposited, becomes more highly crosslinked.

Elevated plasma lipids are known to increase platelet sensitivity (a major source of PAI-1 and anti–α-plasmin) (275). Because fibrin and fibrino-

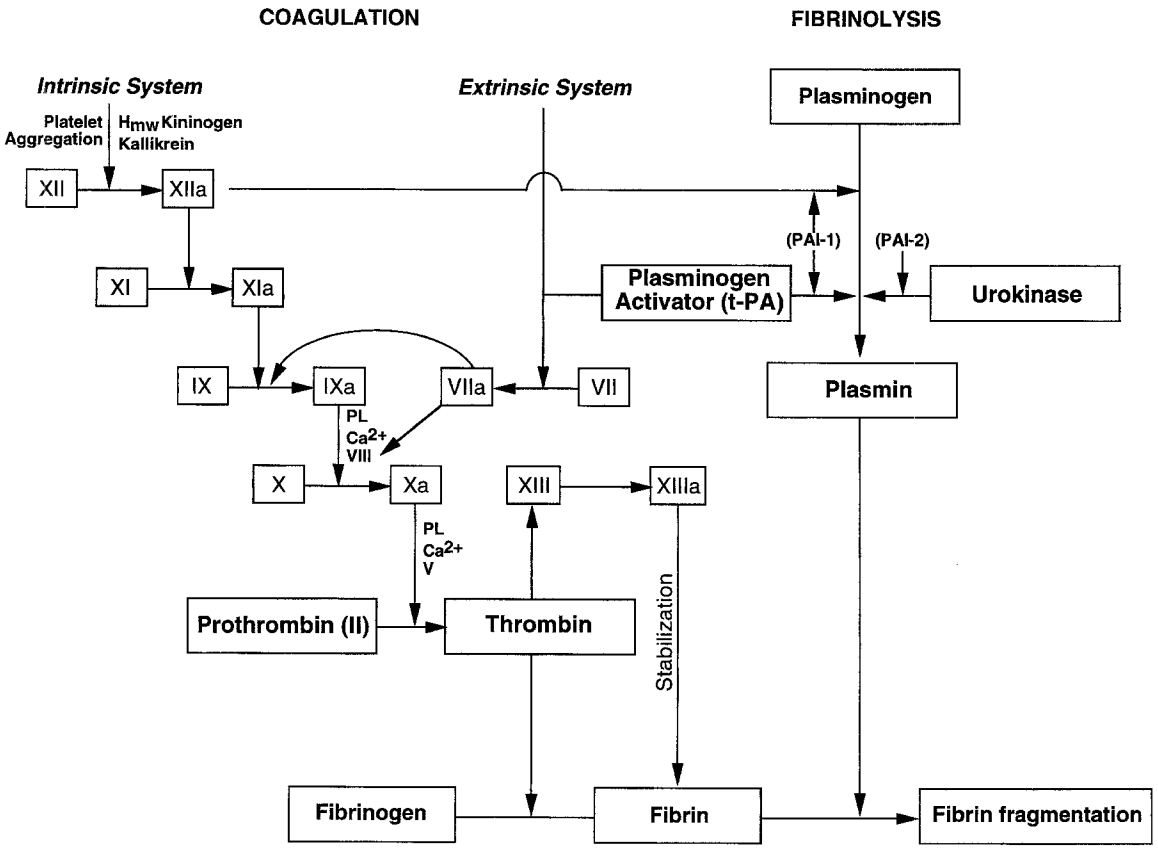

Fig 34. Diagrammatic representation of the major pathways involved in the intrinsic and extrinsic blood coagulation and fibrinolytic systems. Both NaPPS and CaPPs shows weak anticoagulant activity but strong fibrinolytic activity.

gen fragments activate mononuclear cell production of cytokines and other mediators, it is not unexpected that peripheral blood levels of cytokines would be elevated in patients with OA (276). In addition, these active cells promote endothelial and synovial inflammation, thereby contributing to cartilage resorption and inhibition of proteoglycan synthesis by chondrocytes, as has already been discussed. In a recent study, it was shown that the expression of procoagulant activity by mononuclear cells activated by a cartilage-derived antigen (KS-peptides) was elevated in OA patients relative to the non-OA control group (115). This activation of leukocytes by cartilage-derived antigens also releases arachidonate metabolites (eg, thromboxane, PGE_2, leukotriene B_4), and cytokines, and increases expression of adhesion proteins and aggregation of platelets, all of which can directly and indirectly influence the blood clotting and fibrinolytic cascades (277-280).

Blood flow in the subchondral microvasculature also may be reduced by IA effusion because of compression of the epiphyseal venae retinaculares (16, 281). This process would encourage the deposition of thrombi within trabeculae of subchondral bone, particularly if the coagulation/fibrinolytic balance was also disturbed in favor of fibrinogenesis. This scenario is consistent with the observed vascular congestion and increased intraosseous pressure in OA joints, as described previously. The impaired circulation and ischemia in OA joints would be expected to stimulate pain receptors located in the endothelium of the subchondral vasculature bone and synovium, thereby contributing to symptoms.

PPS has profound effects on the blood coagulation, fibrinolytic, and lipid/cholesterol systems, as would be anticipated from its original use as an antithrombolytic/antlipidemic agent. In general, these aspects of the pharmacological activities of

NaPPS and CaPPS result in increased capillary blood flow by promoting lipid clearance and thrombolysis at compromised vascular sites in the elderly, an important activity for the treatment of stroke or in thrombosis after surgery (282-289). A large volume of literature is available to support the hematologic activities of NaPPS, which has been reviewed previously (72, 290) and will not be duplicated here. However, because the rationale for the use of PPS in the treatment of OA is dependent on an understanding of the mechanisms of action of the drug on the fibrinogenic and fibrinolytic systems, a brief description is considered relevant.

PLATELET FUNCTION

Platelet aggregation is pivotal for normal hemostasis and thrombus formation but also plays a significant role in inflammation and tissue repair (291). Platelet adhesion takes place when the endothelial cell surface is disrupted or damaged. Other platelets are recruited to the site of injury by degranulation of the primary platelets, and a hemostatic plug is formed. A variety of compounds (eg, ADP, thrombin, or collagen) then stimulate the platelets to alter their shape and initiate reversible aggregation. A fibrinogen receptor is induced on the cell membrane, and fibrinogen molecules then can cause platelet binding and aggregation. Activated platelets release alpha-granule adhesive glycoproteins, such as thrombospondin, von Willebrand factor, fibrinogen, and fibronectin. These proteins assemble on the platelet membrane and initiate a secondary phase of aggregation, which is nonreversible. The clotting process is then initiated with the formation of thrombin, which promotes additional platelet aggregation and polymerization and deposition of fibrin (see Fig 34).

Studies by Messmore et al (292, 293) on the effects of HEP, low-molecular-weight heparins, DS, and NaPPS on collagen-induced platelet aggregation indicated that NaPPS was less effective than HEP but more potent than other heparinoids in causing aggregation in vitro. This study contrasted with an earlier report by Frandoli et al (294), who showed decreased platelet aggregation with NaPPS when it was administered both orally and parenterally to a group of thrombophilic patients under double-blind conditions. Decreased platelet aggregation by administration of NaPPS also was shown to occur in cats by Schumacher and Classen (295). Vinazzer et al (296) reported that NaPPS increased the maximum collagen-induced platelet aggregation threshold in human plasma ex vivo but had no effect on the lag-time of aggregation or the shape change of the platelets so treated. Dunn et al (297) showed that in vitro NaPPS increased the binding of fibrinogen to ADP-treated platelets more strongly than HEP. Kindness et al (298), although not confirming inhibition of ADP-induced platelet aggregation by NaPPS, showed that the drug inhibited thrombin-induced aggregation.

In a study using 12 normal dogs and 12 dogs with naturally occurring OA, Cheras et al (299) reported that CaPPS when given at 3 mg/kg IM, once per week for 4 weeks, had a significant effect on ECLT and platelet aggregation. Platelets from OA dogs were noted to aggregate more readily in the presence of ADP than platelets of normal animals. However, in animals given the CaPPS for 4 weeks, a reduction in the aggregation threshold in response to ADP was observed after 3 weeks of treatment. This effect lasted up to 4 weeks after terminating CaPPS therapy ($P < .05$).

INHIBITION OF FACTOR Xa

Studies by Soria et al (300) showed that NaPPS showed strong anti-Xa activity both in vitro and in vivo. However, higher concentrations of NaPPS were needed in vitro to inhibit factors Xa than were required in vivo. The inhibitory activity of NaPPS was not demonstrable in the absence of antithrombin III (AT-III), and hence these workers concluded that the inhibition of Xa was AT-III dependent. Similar findings were described by Vinazzer et al (296), who showed inhibition of factor Xa in the presence of AT-III in plasma from patients treated with NaPPS. Czapek et al (301) confirmed that NaPPS could potentiate AT-III inhibition of factor Xa in vitro but also provided evidence that this drug did not cause inhibition by binding strongly to AT-III. This interaction was shown to be concentration dependent by Simmons et al (302), suggesting that NaPPS had a different mechanism of inhibition to that of HEP, which was known to bind strongly to AT-III even at high concentrations.

The study by Thomas et al (303) on the effects of NaPPS and HEP on blood coagulation when administered parenterally to human subjects showed in vivo potentiation of anti-Xa clotting activity as well as the release of lipoprotein lipase. The anti-Xa activity of NaPPS was, however, weaker than that produced by HEP. These results, as well as data

generated by Ryde et al (304) and Soria et al (300), has led to the conclusion that the anti-Xa activity of NaPPS was its major effect on blood coagulation, as opposed to thrombin inhibition. Scully et al (305-307) confirmed that the affinity of NaPPS for AT-III was weak, but its presence could still increase the inhibition of factor Xa. In a later review (308), it was noted that the AT-III and factor Xa interaction could be catalyzed by NaPPS. The apparent dissociation constant of NaPPS for human factor Xa was 20 mmol/L. Using rabbits, Ofosu et al (309) showed that NaPPS was a potent inhibitor of thrombin generation but not a strong accelerator of factor Xa inactivation. Further work by this group (310) showed that PPS had both direct and indirect effects on the inhibition of factor Xa. Not only did the drug directly inhibit factor Xa enzyme activity but it also disrupted the interaction of the constituents of the prothrombinase complex, as well as prothrombin, with coagulant surfaces. From these findings, these authors concluded that inhibition of the catalytic activity of the prothrombinase complex was one of the primary pathways by which sulfated polysaccharides, such as PPS, can act as anticoagulants.

Further in vitro studies to support this interpretation were undertaken by Sie et al (311-314), who provided evidence that the main anticoagulant effects of NaPPS in "normal" plasma were both ATIII and HEP co-factor II (HCII) independent. This would suggest that NaPPS acts by directly preventing thrombin generation by factor Xa rather than by enhancing inhibition of thrombin (Fig 34).

In summary, although earlier work suggested that NaPPS inhibited factor Xa in a similar manner to HEP, that is, by potentiating the inhibition of thrombin by ATIII, more recent studies have shown that NaPPS interferes with the binding of factor Xa to the thrombin by an AT-III–independent mechanism. This process is considered to be one of the primary sites where NaPPS can influence the blood coagulation system, but its activity in this regard is much less potent than that exhibited by HEP.

INHIBITION OF THROMBIN

Thrombin (or factor IIa) is generated from prothrombin (factor II) by the action of factor Xa, factor Va, and calcium ions in the presence of phospholipids (the prothrombinase complex) (see Fig 34). Thrombin catalyzes a number of reactions, including the conversion of fibrinogen to fibrin and the activation of factors V, VII, VIII, XII, and XIII. Inhibition of thrombin thus not only prevents fibrin deposition but also can influence earlier steps in the coagulation cascade (Fig 34).

It has been reported (296, 315, 316) that the anti-thrombin effect of NaPPS was negligible, because it only exhibited approximately 4% of the inhibitory effect of an equivalent amount of HEP. Moreover, Soria et al (300) showed that NaPPS inhibited thrombin in vitro by an AT-III–dependent pathway. Additional evidence that thrombin inhibition by AT-III was potentiated by NaPPS was provided by Czapek et al (301), but again HEP was found to be 10 times more potent as an AT-III accelerator than NaPPS on a weight-for-weight basis. Czapek et al (301) and Fischer et al (317, 318) also showed the potentiation of inhibition of thrombin by NaPPS in the presence of AT-III and showed that it bound to thrombin as well as factor Xa. In the presence of NaPPS, the dissociation constant for the initial complex of AT-III and thrombin was reduced from approximately 2 mmol/L to 61 μmol/L without any change in the limiting rate constant. Though this acceleration of AT-III inhibition was much lower than that achieved by HEP, it approached that required for physiological activity.

Although the effect of NaPPS on the inhibition of thrombin by AT-III was found to be negligible, the drug strongly prevented HEP potentiation of AT-III–dependent inhibition of thrombin by binding to the thrombin molecule itself. It therefore would appear that NaPPS has a greater affinity for the thrombin molecule than HEP, as was shown by its ability to displace thrombin from a HEP-Sepharose affinity column more easily than HEP (319).

INHIBITION OF FACTOR IXa

Factor IXa is generated from factor IX by the action of factor XIa and calcium ions. Factor IXa forms a complex with factor VIIIa, calcium ions, and phospholipid to form the "Tenase" complex, named for its ability to activate factor X (Fig 34).

Although relatively high concentrations of NaPPS are required for the inhibition of thrombin and factor Xa in plasma, at concentrations less than 2 μg/mL, this drug markedly suppressed the intrinsic activation of factor X (318). This effect was shown to be independent of AT-III and was attributable largely to its ability to inhibit factor IXa.

INHIBITION OF THE ACTIVATION OF FACTOR V

When factor V is activated by thrombin, it becomes part of the "Tenase" complex. Sampol et al (320) reported that when 25 to 100 mg NaPPS was administered intravenously to patients, it extended the prothrombin time, accompanied by a decrease in factor V levels. Ofosu et al (309) confirmed this observation and also reported reduced factor V activation by thrombin in the presence of this drug. In an earlier paper, Ofosu et al (321) concluded that the anti-coagulant effect of NaPPS was mediated primarily through its ability to inhibit the thrombin-dependent activation of factor V, thereby inhibiting the formation of the prothrombinase complex, the physiological activator of prothrombin.

INHIBITION OF FACTOR VIII ACTIVATION

As already indicated, factor VIII is activated by thrombin to form factor VIIIa and become part of the "Tenase" complex. One report (304) stated that NaPPS prevented factor VIII activation by thrombin independently of HEP cofactor II (HCII). It is highly likely that factor VIII activation is impaired by NaPPS because of the effect of the drug on thrombin. It has been shown that the activation of factor V and factor VIII by thrombin are necessary for the efficient formation of the prothrombinase complex (309).

INHIBITION OF PLASMIN

Fibrinogen and insoluble fibrin are rapidly degraded by plasmin to soluble fragments (Fig 34). It was reported that NaPPS enhanced the inhibition of plasmin by AT-III (301). However, because this only occurred at very high concentrations (approximately 400 μg/mL), which is not achieved at therapeutic doses, it is unlikely that this inhibition has physiological significance.

RELATIVE ANTI-COAGULANT AND ANTI-THROMBOTIC ACTIVITIES OF NaPPS, CaPPS, HEPARIN AND ITS FRACTIONS

The anti-coagulant activity of CaPPS and NaPPS when given intravenously are similar (78), but they are both much weaker anticoagulants on a molar basis than heparin or the low-molecular-weight or chemically modified HEPs (322). The equivalence of NaPPS and CaPPS with regard to anticoagulant and fibrinolytic activities when given intravenously is lost when the drugs are administered orally. In the study by Klöcking et al (78), in which both drugs were given orally to rats at 5 mg/kg, CaPPS showed a significantly higher activated partial thromboplastin time (aPTT) activity than NaPPS given at the same dose. From these data, it was estimated (78) over the 4-hour experimental period 10% to 20% of CaPPS was orally absorbed, whereas only approximately 1% of NaPPS was bioavailable through this route of administration.

STIMULATION OF FIBRINOLYSIS

There are several pathways for the conversion of plasminogen to plasmin, the enzyme responsible for the degradation of fibrin (see Fig 34). One of the major activities of NaPPS is to stimulate fibrinolysis, which was observed as early as 1962 (323), a finding confirmed by other investigators (294, 315, 324-329).

Marsh and Gaffney (330) were the first to suggest that the shortened ECLT induced by NaPPS might be attributable to the release of t-PA from vascular endothelium. Shorter ECLTs were reported by Fischer et al (317) after subcutaneous injection of NaPPS to human subjects. Barrowcliffe et al (331) also noted that NaPPS induced the release of lipoprotein lipase to a greater extent than after HEP administration, suggesting that the drug had a marked effect on the endothelium. It subsequently was shown that NaPPS increased the cell-associated t-PA activity in bovine endothelial cells (332). Vinazzer (285, 316, 333) showed that administration of NaPPS (300 mg/d) to patients over 12 months failed to diminish the initial enhancement of fibrinolysis caused by NaPPS. It also has been shown that NaPPS acts in vitro on fibrinolysis through factor XII and prekallikrein as well as increasing the availability of t-PA activator by decreasing the levels of the its endogenous inhibitor (PAI-1) (285, 316, 333-336) (Fig 34).

PPS, as the sodium and calcium salts, release t-PA from the isolated pig ear and rat lung preparations when given orally or parenterally (78, 79, 337, 338). In isolated rabbit ear and whole-body studies in rabbits, the t-PA was shown to be of the tissue-type. After both oral and subcutaneous administration to rats or human volunteers, PPS enhanced fibrinolytic activity and reduced levels of its inhibitor PAI-1 in plasma (79, 287, 329, 337, 339). These pharmacological effects were maximal between 2 and 4 hours after drug administration.

As already discussed, NaPPS and CaPPS have been extensively evaluated as a fibrinolytic agents in several experimental models. In the model developed by Breddin and co-workers (80, 340, 341), vascular injury and thrombosis were induced in rat mesenteric venules by irradiation with a defined quantum of laser energy. Animals were given drugs before and after thrombus formation, which was observed microscopically. The number of laser pulses (injuries) given 1 minute apart required to induce thrombus formation in the presence of the drug provided an index of the drug's antithrombotic activity.

In this rat model, CaPPS was a more potent antithrombotic agent than NaPPS when the drugs were administered subcutaneously or orally (80). Although the mechanism(s) responsible for the antithrombotic activity of CaPPS have not been clearly resolved, data from the laboratories of Klöcking et al (79) suggest that this effect may be mediated by enhanced release of t-PA from endothelium by CaPPS and the reduction in plasma PAI-1 levels. Using young (9 weeks) and old (52 weeks) rats, CaPPS given as a single oral dose of 10 mg/kg induced a marked increase in the release of t-PA in plasma that lasted for up to 8 hours (peak, 2 hours) postadministration. From these experiments, it was estimated (79) that CaPPS enhanced net t-PA activity in the plasma of young rats by 50% when given orally at 10 mg/kg. Under the same experimental conditions, NaPPS was less effective.

In a more recent study, Klöcking (334) examined the acute release of t-PA and its endogenous inhibitor PAI-1 in rat plasma after intravenous injection of NaPPS and CaPPS, as well as a variety of other polysulfated polysaccharides, including the low-molecular-weight HEP. Of the 17 compounds examined, NaPPS and CaPPS were among the most potent stimulators of t-PA activity in rat plasma. CaPPS was found to be more potent at 10 mg/kg than NaPPS. Moreover, although NaPPS only slightly reduced PAI-1 activity at this dose, CaPPS lowered plasma levels of the inhibitor by 60%. Because net fibrinolytic activity represents the balance between t-PA and PAI-1, it could be predicted, on the basis of these experiments, that on a weight basis CaPPS would be a more superior antithrombotic agent than the commercially used NaPPS.

The thrombolytic activity of NaPPS relative to HEP also has been examined in rats (342) and dogs (343). Experimental venous thrombosis was induced in jugular and saphenous veins of 54 mongrel dogs by IV injection of sodium tetradecyl sulfate. NaPPS (2.5 mg/kg) was given intravenously 48, 72, and 120 hours later. The number and weight of jugular thrombi formed in the presence of NaPPS was significantly reduced for up to 24 hours posttreatment. NaPPS also appeared to be more effective than HEP in reducing the weight of thrombi that accumulated at the site of sclerosant application. The antithrombotic activity of CaPPS (determined as ECLT) was also clearly shown in another dog study reported by Cheras et al (299), who observed that 3 mg/kg given subcutaneously over 4 weeks reduced ECLT to normal levels for up to 3 weeks after drug treatment. Increased plasma t-PA and reduced PAI-1 levels also were demonstrated in OA patients when CaPPS was given IM at 3 mg/kg (344).

Summation of these findings indicates that both NaPPS and CaPPS have the capacity to mobilize thrombi, which may become localized in the vasculature of subchondral bone and impede blood flow and osteocyte nutrition in OA joints. The thrombolytic activities of NaPPS and CaPPS in OA joints is, therefore, probably mediated by their ability to stimulate the release of t-PA from endothelium as well as reduce the circulating levels of its endogenous inhibitor PAI-1.

ANTI-LIPIDEMIC ACTIVITIES OF NaPPS AND CaPPS

Associated with the hypercoagulability and hypofibrinolytic status of many individuals with OA is a disturbance in fat and lipid metabolism (14, 15, 274, 345). In these patients, infarction of the subchondral vasculature by lipid and fibrin thrombi can lead to necrosis of subchondral bone and bone marrow accompanied by remodeling and repair.

The effects of NaPPS on fat metabolism have been extensively characterized with respect to the increase of the clearing factor and the reduction in blood total lipids, triglycerides, cholesterol, and the lipoprotein ratios (346). Brunaud et al (347) examined the clearing effect of NaPPS on plasma lipids in rats and dogs. A group of four dogs were used and blood collected 15 and 30 minutes, 1, 2, 4, and 6 hours after the IV or IM administration of 25 mg/kg NaPPS. As shown in Figure 35, it is evident that after drug administration, plasma lipids decreased relative to non–drug-treated controls for at

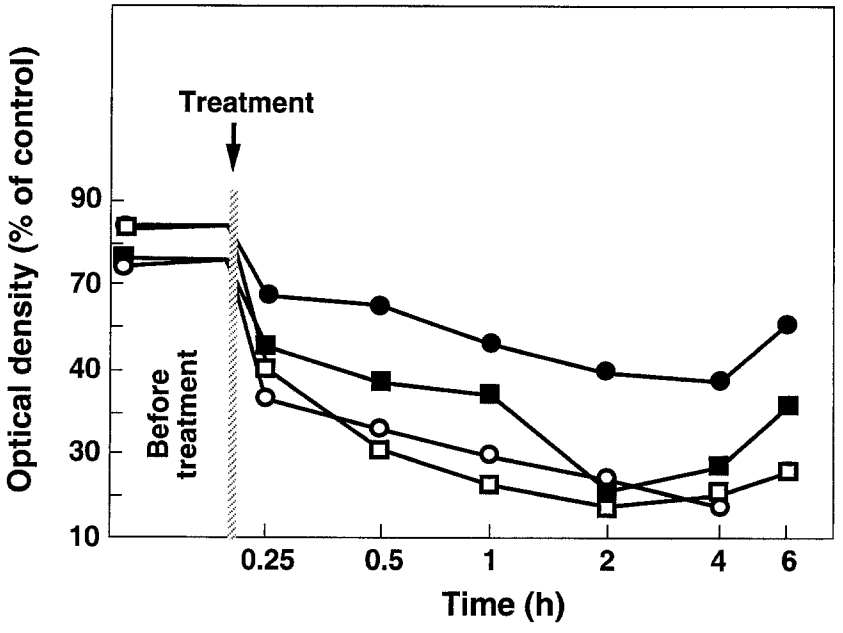

Fig 35. Clearing (lipase-stimulating) effect of NaPPS on plasma lipids in four dogs over 6 hours when the drug was administered intramuscularly at 25 mg/kg □ Dog A; ○ Dog B; ● Dog C; ■ Dog D. (Data from Brunaud et al [347].)

least 6 hours. HEP, when given at doses high enough to produce a full clearing effect, also inhibited blood clotting (347). However, equivalent doses of NaPPS, which achieved a similar level of plasma clearing, did not alter blood coagulation in this model. Raveux et al (348) also fed rats a high-fat/high-protein diet over 7 months to produce increased plasma levels of total fats and a change of lipoprotein pattern in favor of the β-fractions. NaPPS (5 mg/kg intraperitoneally [IP]), as well as HEP (5 mg/kg IP), administered daily cause regression of the lipid changes induced by the 7-month high-fat diet. Three weeks of therapy with NaPPS almost normalized the total blood lipids and restored the lipoprotein pattern, whereas control animals remained unchanged. The qualitative effects of HEP and NaPPS were similar, but it was concluded that, under the test conditions used, NaPPS was superior to HEP because of reduced potential for bleeding (347, 348). However, histological and chemical studies of deposits of cholesterol and neutral fats in organs from these experimental animals also showed that NaPPS achieved disintegration of deposits to a higher extent than HEP (347).

More recent studies using the dog (274, 349) have confirmed the ability of CaPPS to increase plasma levels of lipases and modify other hemodynamic parameters. Oral gavage of CaPPS at 10 mg/kg to dogs stimulated a rapid release of hepatic lipase and lipoprotein lipase into plasma within 2 hours of administration (349). Superoxide dismutase (SOD) also was increased in plasma after this protocol, but maximal levels were achieved 2 to 4 hours after CaPPS treatment. Elevated plasma levels of SOD in fasted rabbits also has been reported (81) when the drug was administered orally at 10 mg/kg (Fig 36). In the fasted rabbit, rapid absorption of CaPPS occurred (probably from the stomach), because SOD was released into the circulation within 1 hour of administration (Fig 36). Elevated levels of free fatty acids in plasma of thoroughbred horses after IV administration of NaPPS (1.3 mg/kg) has been shown (350).

The mechanism of action of NaPPS in accelerating intravascular lipolysis appears to be by activating (or releasing) lipases, including the glyceryl-ester hydrolase system (tri, di, and monoesterases) from the endothelium and liver (351). However, other mechanisms, including action of the drug on platelets and release of inflammatory mediator, also may be contributory (282, 283, 352).

The lipolytic effects of NaPPS also have been confirmed in women (n = 11) with mild to moderate OA (353). A single IM injection of NaPPS (3 mg/kg) was given and blood collected at 0, 1, 2, 4, 8, and 24 hours after drug administration. Plasma was isolated, and lipoprotein lipase and hepatolipase determined using standard assays. In addition, plasma levels of SOD were assessed using a

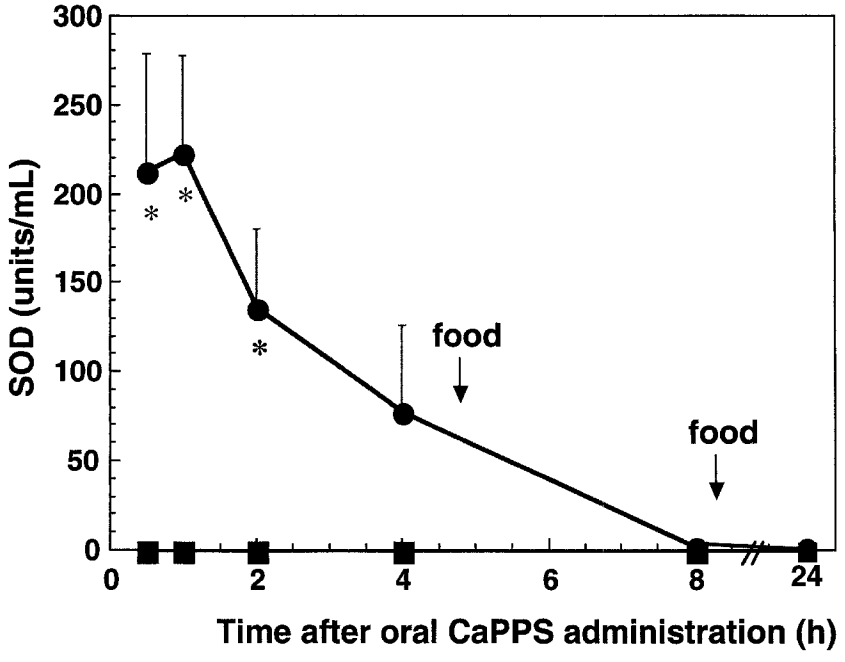

Fig 36. Mean (±SEM) levels of superoxide dismutase (SOD) activity in rabbit plasma obtained after a single oral dose of CaPPS (10 mg/kg) after overnight fasting (●). Levels of SOD relative to levels in plasma of a non–drug-treated group (■). The elevation of SOD was significant for up to 2 hours post-CaPPS administration. *$P < .05$.

commercially available kit. The results of these investigations are summarized in Figure 37, where it is evident that at the IM dose of 3 mg/kg, NaPPS had a marked effect on the levels of both lipases and SOD in patients' blood, producing maximal plasma levels between 2 and 4 hours after administration.

These same patients also were used to determine the action of NaPPS on peripheral blood mononuclear cell procoagulant activity (MPA) and individual leukocyte populations before and 24 hours and 4 weeks after a 4-week course of IM treatment (3 mg/kg) of the drug (115, 354). In this group of women (average age, 62 years; average body mass index, 33), MPA, which before treatment was higher than normal, was significantly reduced to within the normal range after a single IM injection of NaPPS. After four once-a-week injections of NaPPS, the decreased MPA was maintained for a further 4 weeks but only in those patients with moderate OA (Kellgren and Lawrence OA grade 2). Concomitant with decreased MPA was increased number of blood leukocytes. Although peak blood drug levels occurred 4 hours postinjection (Fig 38A), increased leukocyte numbers persisted for 8 hours or more but returned to baseline by 24 hours posttreatment (Fig 38B). Analysis of the types of white cells recruited into the circulation by NaPPS showed that lymphocytes were the predominant population present (Fig 38C). Moreover, 4 weeks after the last injection of NaPPS, lymphocyte numbers were still elevated in the blood of 9 of 11 patients relative to the baseline values (115). It was suggested that this effect of NaPPS on lymphocyte distribution could arise from the interaction of the drug with receptors on the lymphocytes or postcapillary endothelial cells, which normally regulate leukocyte recirculation through lymphoid tissues (115).

The clinical response of these limited number of OA patients to NaPPS treatment over a 24-week assessment period was positive, notwithstanding the unblinded design of the study. Patient overall evaluation of effectiveness of therapy showed significant improvements from baseline levels by the end of the third week ($P < .05$) of NaPPS treatment. This improvement was maintained through the 12th week ($P < .005$) but thereafter gradually returned to pretreatment levels. Improvement in global pain showed a complementary pattern. Significant improvement in morning stiffness using the visual analog scale (VAS) was achieved 8 weeks after initiating therapy ($P < .05$). A marked decline in pain at rest or walking was noted after NaPPS treatment, with maximum effects occurring by week 8 ($P < .005$). However, the improvement in walking pain persisted until week 15 of the study ($P < .05$).

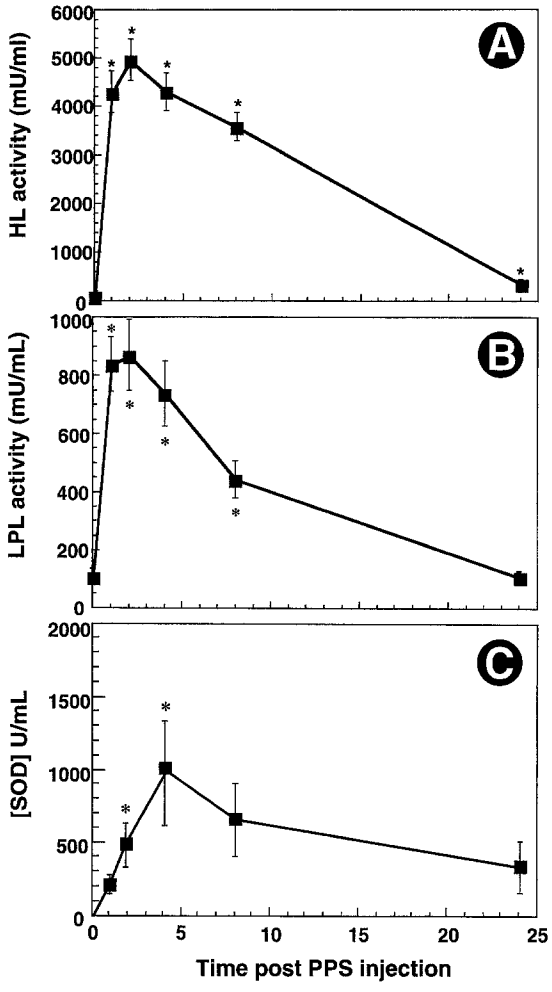

Fig 37. Mean (±SEM) levels of hepatolipase (HL) (A), lipoproteinlipase (LPL) (B), and superoxide dismutase (SOD) (C) measured over 24 hours in women with knee and hip OA (n = 11) after a single intramuscular injection of NaPPS (3 mg/kg). *Significant change from baseline values ($P < .05$) occurred in all parameters for 4 hours after drug treatment but lasted for 8 hours for lipase activities (A) and (B).

For patients with moderate OA, correlations were observed up to week 8 between the change from baseline in four indices of OA disease activity and their respective MPA values. These clinical parameters included the patients' global assessment of pain ($r = .958$); patients' evaluation of treatment effectiveness ($r = -.915$); VAS pain on walking ($r = .800$); and time to climb 15 steps ($r = .768$). No correlations were observed between MPA and the clinical data generated from patients with mild OA (354).

This limited clinical/laboratory study with NaPPS in human OA patients was in agreement with the outcome of a similar investigation undertaken in 12 OA dogs (274, 299). In this study, correlations were reported between improvement in symptoms after CaPPS subcutaneous treatment (3 mg/kg) and correction in abnormalities in ECLT and threshold for ADP-induced platelet aggregation. Cheras et al (274) have proposed that the imbalances in fibrinogenesis, fibrinolysis, and lipid metabolism observed in the plasma of patients with OA could be used as surrogate markers of disease activity.

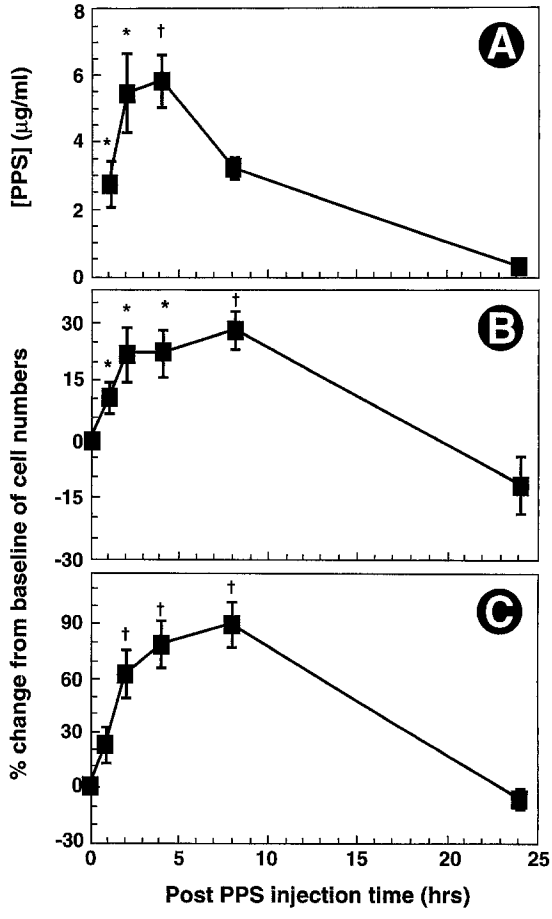

Fig 38. Plasma levels obtained in OA women (n = 11) after a single intramuscular injection of NaPPS (3 mg/kg, A) and the effects of this dose on the change from baseline of their total leukocytes (B) and lymphocytes (C). *$P < .05$, †$P < .005$. (Data from Anderson, Edelman, and Ghosh [115].)

CLINICAL STUDIES WITH NaPPS AND CaPPS FOR SYMPTOMATIC RELIEF IN OA

Veterinary Clinical Studies

The veterinary clinical use of NaPPS and CaPPS for the treatment of OA and related disorders in the dog is now established as safe and efficacious. NaPPS as an injectable formulation was first registered for use in the canine in Australia in 1986. The drug was subsequently approved for use in the treatment of canine OA in New Zealand, Finland, the United Kingdom, Canada, and Eire. Registrations are also pending in other EU countries. During this time, many millions of injections using the recommended systemic dose of 3 mg/kg once per week for 4 weeks have been given with very few reported side effects or adverse reactions. Furthermore, in a recent review of preferences of Australian veterinarians for different antiinflammatory preparations for the treatment of canine arthropathies, NaPPS (as Cartrophen; Arthropharm Pty Ltd, Sydney, Australia) emerged as the most favored treatment option (355). Most of the clinical studies of NaPPS that were used for the registration of NaPPS and CaPPS were company-sponsored and therefore are not in the public domain. However, two studies have been published.

In the study by Read, Cullis-Hill, and Jones (356), injectable NaPPS was evaluated at three different doses to determine efficacy and safety relative to placebo over a 2-month period. Forty geriatric dogs with well-established clinical signs of chronic OA were treated on four occasions at weekly intervals with either placebo or 1 mg/kg, 3 mg/kg, or 5 mg/kg of NaPPS by SC injection. The 3-mg/kg dose gave consistently significant improvement in lameness and other clinical scores of disease activity relative to the placebo-treated groups. The 1-mg/kg dose was only partially effective, but the 5-mg/kg dose was least effective, sometimes causing joint pain, which probably was due to the known effects of NaPPS on the release of kinins when used at high dose (214, 216). No adverse side effects were reported during the trials.

The second study was a randomized prospective clinical trial comparing surgery versus NaPPS treatment of fragmented coronoid process and osteochondritis dissecans of the canine elbow joint (357). Nineteen dogs were randomly assigned to either surgery or NaPPS treatment (3 mg/kg SC once per week for 4 weeks). Assessment of treatments was determined by force plate analysis, gait evaluation, joint palpation, range of motion, and radiographic criteria before and 2, 4, 6, and 9 months after treatment. Ten dogs received surgical treatment, and nine dogs NaPPS. On completion of the study, the lameness score had improved in 70% of the surgical group and 56% of the NaPPS group, but these differences were not significant. The extent of elbow joint pain and range of motion improved ($\approx$67%) equally in the two groups, but radiographic score of degenerative changes decreased in both groups 9 months after treatment. Force plate analysis showed that the mean vertical force (MVF) in the more affected limbs of the surgical group was significantly lower than the mean MVF for the NaPPS group at 2 months. At 4 months and for the remainder of the 9-month study, there was no significant difference with respect to mean MVF in the more affected limb. Although the study failed to show significant differences in outcomes between surgery and NaPPS treatment of elbow arthropathy, the relative financial costs were significantly different, suggesting that NaPPS could represent a more economical alternative to surgery for treatment of this common canine problem.

Human Clinical Trials

The use of PPS as the sodium and calcium salts has been reported in four controlled clinical trials of patients with OA. Edelman et al (358) performed a double-blind placebo-controlled study to evaluate the effect of IM NaPPS (3 mg/kg once per week for 4 weeks) on the symptoms of OA patients over a 6-month assessment period. One hundred fourteen patients were recruited (60 placebo and 54 NaPPS treated) and were well matched apart from duration of symptoms (placebo, 6.8 ± 0.7 years; NaPPS, 5.0 ± 0.5 years; $P = .03$). After a 2-week NSAID washout period, baseline clinical assessment of pain at rest, pain on walking, and early morning stiffness were determined using a VAS. Step time (minutes), early morning stiffness duration (minutes), treatment effectiveness, global pain assessment, and lifestyle function score based on the Western Ontario and McMaster Universities osteoarthritis score (WOMAC [359]) were determined. Clinical assessments were also made during the 4-week NaPPS injection period, then 2, 3, 4, and 6 months from the commencement of the study. Clinically important improvement was defined a priori to identify responders. A 2 cm or greater

change in VAS for stiffness, pain at rest, or walking was considered significant. In addition, the outcome of assessments were expressed as a change from baseline for NaPPS and placebo groups after each visit and the difference analyzed statistically. Although all 114 patients completed the 4-week course of treatments, only 32% of the placebo group and 61% of the NaPPS group completed the 6-month follow-up (difference, $P < .025$). Significant differences between PPS and placebo groups were found for five of the seven clinical parameters used. Stiffness, pain at rest and walking, patient assessment of effectiveness of treatment, and several lifestyle scores (individually and in groups) showed significant improvement in the NaPPS-treated group relative to the placebo group. Investigators concluded that four consecutive weekly IM injections of NaPPS (3 mg/kg) significantly improved the symptoms of OA for up to 2 months and some parameters for 5 months posttreatment. No adverse side effects were reported during the trial.

In an open study of 23 patients with mild to moderate OA of the hand, hip, or knee, Verbruggan et al (82, 344) reported that CaPPS (2 mg/kg intramuscularly once a week for 5 weeks) provided symptomatic relief and modified biochemical and hematologic markers of blood coagulation and thrombolysis. Blood samples were collected 1, 2, 3, 4, and 24 hours after the first CaPPS injection and then on subsequent visits of 2, 3, 4, 8, 12, and 16 weeks. Clinical assessment of VAS scores for walking, pain, pain at rest, pain at night, and functional indices for fingers, hips, and knees were determined individually and as combined scores. Peak CaPPS levels of 5.34 $\pm$ 0.40 μg/mL were achieved in plasma 4 hours postinjection. By 8 hours, they had declined to 1.70 $\pm$ 0.18 μg/mL and CaPPS was negligible after 24 hours. The thrombin and aPPT were significantly elevated, and the quick time decreased, within 2 hours of drug administration. Plasminogen activator levels in plasma were elevated after 8 and 24 hours, whereas PAI-1 was decreased from 1 to 8 hours after CaPPS injection. However, mean PAI-1 levels were decreased and α1-antitrypsin inhibitor levels increased over the duration of the trial. Clinical evaluation using all 23 patients showed that there was a significant (paired samples) reduction of the VAS global pain after 2 weeks (after two injections) ($P < .008$), which persisted for the 16-week trial period ($P < .0003$) compared with baseline. For the finger joints, only the pain reduction was significant after 8 weeks ($P = .03$) and lasted until weeks 8 ($P = .03$) and 16 ($P = .06$). For knee OA, the reduction in pain was significant after 8 weeks ($P = .03$) and persisted to week 16 ($P = .04$). Patients with more severe hip OA did not show significant improvement before the end of the study. However, if hip and knee patient data were pooled and analyzed together, a significant reduction in VAS pain was evident over the entire evaluation period. Significant improvement in functional scores were observed for knee ($P = .03$) and finger OA ($P = .02$) but not hip OA over the duration of the study.

CaPPS also has been studied when given orally to patients with OA of the finger joints under double-blind, placebo-controlled conditions using a broken treatment protocol (360). Fifty patients were recruited (24 CaPPS-treated, 26 placebo-treated), after a 3-week washout period in both groups. CaPPS or placebo capsules were randomly given at a dose of 20 mg/kg PO twice a week for 6 weeks; then a 6-week nontreatment period was allowed to elapse. A second course of drug or placebo treatment was then initiated using the same dosage schedule as used in the first course. The study was terminated 6 weeks after the last week of the second course of treatment, that is, 24 weeks since commencement. The patients were examined clinically before entry and 3, 6, 9, 12, 15, 18, 21, and 24 weeks thereafter. The clinical parameters evaluated included VAS global pain, pain on heavy finger activities, pain on light finger activities, pain at night, morning stiffness, grip strength, the "Ghent" functional index for fingerjoints, and the Ritchie Index (pain on palpitation and count for analgesic drugs). Data were analyzed as change from baseline for the entire period and for the second treatment period only, that is, weeks 15 through 27.

This study showed significant improvement after the second course of treatment in global pain, morning stiffness, pain at night, dysfunction while conducting heavy and light daily hand activities, the functional index for finger joints, and pain on palpitation. Grip strength and consumption of analgesics were not different between CaPPS and placebo groups. Although the placebo response was initially strong, it diminished significantly in the last 3 months of treatment and never reached a 20% change from baseline in any parameter. In contrast, the same assessments for the CaPPS-treated group

all showed a 20% or greater improvement relative to baseline by the end of the trial, that is, for 6 weeks after the last course of the therapy.

These data strongly suggest that multiple courses of treatment with CaPPS may be more effective in providing symptomatic relief than a single loading dose. From this study, Verbruggen et al (360) concluded that CaPPS at 20 mg/kg produced symptomatic relief in patients with OA of the hands for up to 6 weeks after cessation of treatment without any apparent side effects.

IA NaPPS also has been evaluated under double-blind conditions against an equivalent volume of IA Ringer's solution in a group of patients with OA of the knee. Eighty-six patients were enrolled in the study, but the interim report (361) only described the analysis of 15 NaPPS- and 16 placebo-treated patients. Rigorous entry requirements selected only those with unilateral OA or clearly separable bilateral joint disease, with one joint having Kellgren OA grades of 2 to 3. Walking pain was required to exceed 2 cm on a 10-cm VAS scale, and those who had received other IA therapies or knee trauma previously were excluded. After a 2-week NSAID washout period, patients were assessed at baseline for time to walk 50 feet (t50), VAS of maximum pain (VAS max) during the previous 48 hours and previous month, WOMAC (359) scores for pain, stiffness and functional deficits, global assessment of treatment effectiveness, range of motion, crepitus, joint diameter, and effusion. Patients were randomly assigned to a course of 4 weekly IA injections of either 1 mL Ringer's solution (placebo) or 1 mL NaPPS (50 mg) allocated under blinded conditions. The clinical status of each patient was determined at the time of each injection (weeks 0, 1, 2, 3) and on weeks 4, 5, 9, 12, 16, 21 and 26 thereafter. Synovial fluid (SF) also was aspirated at each of the visits before giving the four weekly IA injections and was analyzed for osteocalcin, MMP-3, TIMP, and nitrite levels. SF viscoelasticity and HY molecular weight were also assessed using a novel microrheometer (260). Weekly pain indices (time, VAS) and functional scores (VAS) were expressed as change from baseline (week 0) for each patient. In the placebo-treated group, clinical improvement from baseline was observed. However, in all cases, the NaPPS-treated group showed greater significance than the placebo group for the same indices. In addition, significant differences between the NaPPS-treated and placebo groups were observed for stiffness (weeks 5, 6, 21, $P < 0.05$), walking on a level surface (weeks 9, 12, 16, 21, $P < .05$) and pain in the previous 48 hours (weeks 9, 12; $P < .02$) and previous month (weeks 4, 5, 9, 12, 16, $P = .01$ to .04).

Analysis of SF for MMP-3 and TIMP failed to show any correlations with treatments. However, in the NaPPS-treated groups, osteocalcin levels diminished relative to baseline on weeks 1 ($P = 0.03$), 2 ($P = 0.02$) and 3 ($P = 0.02$), whereas the placebo group levels remained constant and were higher than NaPPS on week 2 ($P = .05$) and 3 ($P = 0.06$). Nitrite levels (the metabolite of NO·) in SF were lower in the NaPPS-treated group than in the placebo group but only on week 3 ($P = .001$).

The authors concluded that the NaPPS group showed significant improvement in pain and mobility indices for up to 2 months after completing the treatment protocol. Because SF viscoelasticity (260), osteocalcin (a marker of osteoclast activity and bone remodeling), and nitrite levels in SF of NaPPS patients were lower than in the placebo group (361), the clinical benefits of the drug might be related to pharmacological modification of synovial inflammation and synoviocyte metabolism.

SUMMARY AND CONCLUSION

In this review, laboratory and clinical studies have been described that show clearly that NaPPS and CaPPS show a range of pharmacological activities that are relevant to metabolic pathways implicated in the pathobiology of OA. The major sites of intervention of these drugs are identified by arrows in Figure 1, but may be summarized as (1) preservation of cartilage integrity by supporting chondrocyte anabolic activities, including biosynthesis of aggrecan and downregulating or inhibiting proteinases responsible for matrix catabolism; (2) reducing synovial inflammation, complement activity, and release of pro-inflammatory mediators by leukocytes and synoviocytes; (3) cytoprotection of synoviocytes and normalization of their ability to synthesis high-molecular-weight HY; (4) improving blood flow in synovium and subchondral bone by stimulating release of t-PA, SOD, and lipase from capillary endothelium, thereby mobilizing fibrin and lipid occlusions; and (5) increasing the threshold for activation of platelets and release of PAI-1.

Controlled clinical trials using NaPPS or CaPPS

in OA dogs or patients have shown symptomatic relief relative to placebo under blinded conditions. Moreover, in some instances, drug-induced clinical improvement in symptoms has shown correlations with surrogate markers of disease activity, such as increased viscoelasticity of SF, a reduction in ECLT, or decrease in mononuclear cell procoagulant activity. These data, together with other studies collected in this review, provide support for the contention that NaPPS and CaPPS should be considered as SMOADs. However, additional long-term clinical studies in conjunction with methods of monitoring joint structural changes in OA patients are required to confirm this supposition. Because CaPPS has shown superior bioavailability to NaPPS when given by the oral route, it offers clear advantages over NaPPS for such long-term investigations.

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