There's News in Osteoarthritis – From NO to COX-2

"What are those hard little knots, about the size of a small pea, which are frequently seen upon fingers, particularly a little below the top near the joint? They have no connection with the gout, being found in persons who never had it, they continue for life; and being hardly ever attended by pain, or disposed to become sores, are rather unsightly than inconvenient, though they must be some little hindrance to the free use of the fingers." – William Heberden, M.D., Commentaries on the History and Cure of Diseases, 1803.

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Interests:

- Lymphocyte function in autoimmune diseases
- Role of oxidative stress in control of lymphocyte activation
- Novel therapies for arthritis

Abbreviations used:

OA ..... Osteoarthritis
RA..... Rheumatoid Arthritis
BMI..... Body Mass Index
BMD .....Bone Mineral Density
IL-1, -4, etc. ..... Interleukin 1, 4 etc.
TNF-α..... Tumor Necrosis Factor α
TGF-β ..... Transforming Growth Factor β
EGF..... Epidermal Growth Factor
PDGF..... Platelet-Derived Growth Factor
FGF.....Fibroblast Growth Factor
MMP .....Matrix Metalloproteinase
TIMP .....Tissue Inhibitor of Metalloproteinase
NO.....Nitric Oxide
NOS..... Nitric Oxide Synthase
COX..... Cyclooxygenase

INTRODUCTION

Osteoarthritis (OA) is the most prevalent musculoskeletal condition. It has been estimated that 12.1% of Americans over the age of 25 have clinical evidence of OA (1). This translates to 21 million persons in 1998. Radiographic OA is present in the knees of up to 33% of persons 63 and older, a population group that is expected to increase by 82% in the next twenty-five years (2).

OA is often overlooked as a serious medical condition, owing in part to its common nature and to the typical slow progression of the disease over several decades. The abandoned term "degenerative joint disease" (DJD) implies an inevitable wearing-out of the joint – a natural consequence of aging. It is now clear that many older individuals do not have OA, offering hope for medical intervention. The term "osteoarthrosis" was proposed to indicate the non-inflammatory nature of OA in contrast to rheumatoid arthritis (RA). With some of the studies that will be presented in this Grand Rounds, the important, yet distinct, contribution of inflammatory mediators to the pathogenesis of OA is obvious – making it a true "-itis".

OA has a considerable economic and social cost. It is second to chronic heart disease as the diagnosis leading to Social Security Disability payments (3). In an analysis of older persons in the Framingham study, OA of the knee was listed as the leading cause of dependence on others for mobility within the home and in the community. In the Longitudinal Study on Aging in USA, 55% of persons age 70 or more were found to have OA. 78% of these people had reduced physical activity and 36% reported a decrease in their ability to carry out activities of daily living. Both medical and non-medical costs of OA have also been estimated by Gabriel, et al. (4, 5). Using a sample of the Rochester Epidemiology Project at the Mayo Clinic, they determined that the median medical costs (1987 dollars) of RA, OA and age and sex-matched controls were $1,050, $667, and $232, respectively. Indirect and non-medical costs (excluding wages) for these groups were (1992 dollars): $890, $726, and $335. Moreover, 10.5% of the OA patients reduced their work hours or stopped working due to their arthritis, 13.7% retired early due to OA, and 10.3% reported a drop in household income due to arthritis. While the costs for RA were higher, its prevalence is much less (0.8% and declining). Thus, the excess costs (ignoring inflation and wage losses) exceed $17 billion. These trends are seen in all developing countries studied including the US, Canada, the UK, France, and Australia where costs associated with OA have been estimated to consume 1-2.5% of Gross National Product (6).

It is impossible to review all the recent basic and clinical research relating to OA in a single Grand Rounds. The following topics will be addressed:

- Epidemiology, genetics, and identification of risk factors for OA in an effort to prevent its occurrence
- Review of current concepts in the biology of cartilage, the pathologic tissue in OA
- Evidence for altered chondrocyte metabolism as a central feature of OA. This is manifest primarily by the production of inflammatory mediators and offers possibilities for novel therapeutic interventions.
- A discussion of the merits of selective COX-2 inhibition in the treatment of OA

EPIDEMIOLOGY

Assessment of the prevalence of OA is hampered by the lack of a consistently applied definition of the condition. OA can be diagnosed radiographically by the presence of osteophytes, joint space narrowing, sub-chondral sclerosis, and deformity. Standard radiographs have been published that grade OA on a scale of 0-4. Using these scales, a number of population-based, cross sectional studies have estimated the prevalence of OA (grade 2-4) in men and women of different ages. Typical data are shown in Figure 1, where the prevalence of OA in an entire Dutch region of ~6,500 persons was determined (7).

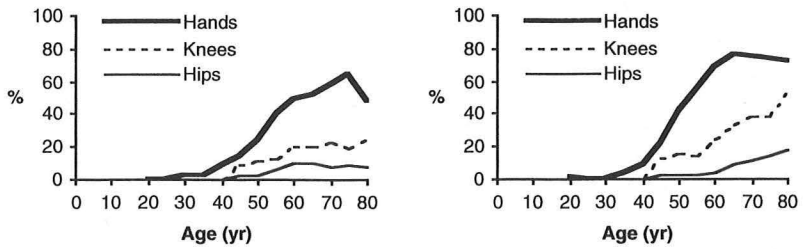

Figure 1 Radiographic prevalence of osteoarthritis in men (left panel) and women (right panel) in the Netherlands 1975-78 (7).

However, radiographs tend to overestimate the prevalence of clinically significant disease. Problems with technical reproducibility make longitudinal studies or multi-center studies difficult. There are fewer studies that look at symptomatic OA, or OA diagnosed by clinical exam confirmed by radiographs. The Framingham Osteoarthritis Study has provided some of the most detailed data in this regard, owing to the long-term follow-up of identified patients, and consistency in the analytical methods. Their most recent data suggest that symptomatic knee OA (defined as pain on most days and a characteristic x-ray) occurs in 6.8% of men and 12.2% of women over age 30. Symptomatic OA of the hips occurs in 5.5% of men and 3.6% of women over age 55. The criteria developed by the American College of Rheumatology for the OA of the hand, knee, and hip take into account both clinical criteria as well as radiographic evidence of disease.

In all studies, the incidence and prevalence of OA increases with age. OA rarely occurs before age 50. When it does it is slightly more common in men. After age 50, OA is more common in women, suggesting a role for post-menopausal hormonal changes in the pathogenesis. Clear data on racial differences in the incidence of OA are lacking, although some studies have suggested lower rates in blacks living in the Caribbean and Africa, as well as Chinese in Hong Kong when compared to Europeans.

GENETICS OF OSTEOARTHRITIS

The familial occurrence of OA has been noted since the 1940's when Stecher demonstrated that Heberden's nodes were three times more common in sisters of affected patients than in the general population (8). In the 1950's, the clinical entity, "generalized osteoarthritis" was described (9). This condition, common in elderly women, consists of Bouchard's and Heberden's nodes of the hands along with polyarticular OA of the spine, knees, and hips. 36% of first-degree relatives of men with OA, and 49% of the relatives of women with generalized OA also developed disease, compared to population figures of 17-26% (10). A number of twin studies have been done in OA. Nodal OA develops in 60% of monozygous co-twins of probands versus 13% with a normal co-twin. 39% of dizygous co-twins develop nodal OA (11). A more recent study of 500 female twin pairs aged 45-70 found concordance of radiographic OA of the hand or knee in mononzygotic twins to be twice that of dizygotic twins. Overall, the independent contribution of heritability to OA from these studies was estimated at 39-65%. Additional data from the Framingham Study suggested a correlation of 0.1 to 0.3 for family members with generalized OA. Statistical analysis was most compatible with a mixed model consisting of a recessive Mendelian gene and a residual multifactorial component (12).

Perhaps the most interesting genetic studies on OA have been performed in the laboratories of Moskowitz and Prockop. They have described a number of extended families with mild chondrodysplasia and development of typical findings of OA in fingers, hips, knees, shoulders, wrists, and hands (13). Affected family members inherited a specific restriction fragment length polymorphism in the gene for type II procollagen (*COL2A1*). Sequencing of the gene documented a single base substitution resulting in the substitution of cysteine for arginine in the triple helical region of the protein (14). The presence of the mutation resulted in autosomal dominant expression of disease with 100% penetrance in persons over age 22 (Figure 2).

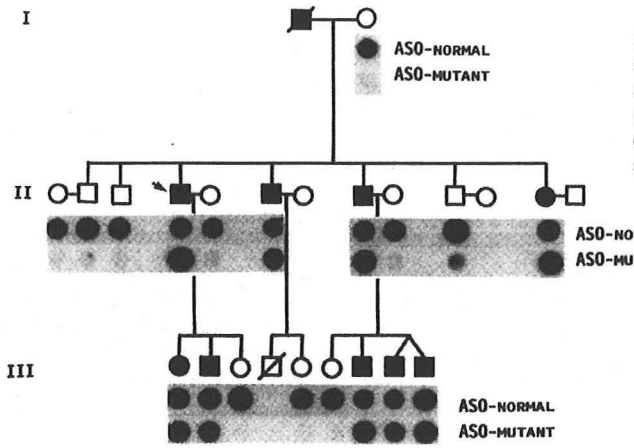

Figure 2 The presence of a C→T substitution detected by the binding of an allele-specific oligonucleotide (ASO) correlates with the inheritance of precocious osteoarthritis (filled symbols) (14).

It is postulated that the presence of the abnormal type II α chain somehow disrupts the structural integrity of the collagen fibril. Whether this is due to formation of disulfide bonds not normally found in collagen or the loss of positive charge from the arginine is not known. Several other mutations of the type II collagen gene have been associated with premature OA, although there is no evidence to suggest that structural defects in type II collagen are responsible for common forms of OA.

RISK FACTORS

The goal of epidemiological studies is to identify risk factors for the prevention or amelioration of disease. Obviously, age, gender, and genetic predisposition cannot be altered, but they may identify persons at high risk. A large number of cross-sectional population-based studies have been performed to look at other risk factors. Most of these studies have looked at disease prevalence, and a few at incidence.

Body Weight

Numerous studies have shown that adiposity is associated with development of OA of the knee, particularly in women (15-17). One of the first cross-sectional studies used data from the Framingham Cohort on weight at the first examination in 1948-1952 and compared that to the incidence of radiographic OA of the knee in the following 36 years (16). The age-adjusted relative risk for any radiographic knee OA was 1.51 for men and 2.07 for women in the heaviest quintile at study entry. The effect of weight was more pronounced for severe radiographic OA.

	Relative risk in heaviest quintile	
	Men	Women
Asymptomatic radiographic OA	1.27	2.30
Symptomatic radiographic OA	2.79	1.64
Severe radiographic OA	1.86	3.16

These persons did not have radiographs at their first examination. Therefore the presence of OA prior to the development of obesity could not be formally excluded. Subsequently, 598 persons whose radiographs did not show OA of the knee were evaluated eight years later with repeat x-rays (18). The average age at the beginning of the study was 70, 63% of them were women, and the mean body-mass index (BMI) was 25.9. Again, BMI at baseline had a strong effect on the subsequent development of knee OA with a relative risk of 1.6. There was a 40% increase in the incidence of OA for each 10 pound weight gain in eight years and a similar decrease in the risk of OA for weight loss. The effect of weight loss confirmed an earlier analysis of the Framingham population that documented a 50% decrease in the risk of developing knee OA with a 2 unit decrease in BMI (~11 pounds) over a 10 year period (19). A separate study confirmed these results (20) in a cohort of 715 English women. The odds ratio for the development of incident OA over a 4 year period was 2.38 in the top tertile of women in terms of BMI (>26.4).

Weight has inconsistently been associated with OA in other sites such as the hip and the hand (21-24). The mechanism leading to increased OA in overweight persons is not

known. Clearly the increased mechanical forces on weight bearing joints (patellofemoral forces are 2-3 times body weight during walking) play a role. However, production of hormones by adipose tissue, or differences in growth factor levels, susceptibility to cytokines, and other metabolic explanations are also possible.

Osteoporosis

Osteoarthritis is negatively associated with osteoporosis in the vast majority of cross-sectional population studies performed (25). For example, 579 women who were part of a population study of OA in Chingford, England had bone mineral density (BMD) determinations in their lumbar spine and femoral neck. Women with radiographic evidence of OA in their hands, spine, hips, or knees all had BMD with increases ranging from 2.5% – 9.3% compared to non-OA controls (26). Multivariate analysis revealed an odds ratio of 2.13 for women in the top tertile of BMD that persisted after correction for smoking, alcohol or estrogen use, exercise, and spinal osteophytes. In a larger study of 4,855 Caucasian women age 65 or older, the 579 women with mild to moderate OA of the hip were found to have age-adjusted BMD values 10% greater than controls in the femoral neck, 4% greater in the lumbar spine, and 5% greater in the distal radius (27).

The reason(s) for this inverse association are not clear. Both OA and BMD are associated with obesity. Controlling for BMI does not appear to alter the relationship. It has been suggested the greater deformation of subchondral bone protects articular cartilage in persons with lower BMD. The appearance of higher BMD at uninvolved sites distant to the joint with OA suggests that it may play a primary role in the pathogenesis of the disease. There is evidence of abnormal metabolism in the subchondral bone in OA, with increased gelatinase activity (28), as well as increased synthesis and mineralization of collagen in cancellous bone of OA patients (29). A 3 – 4 increase in TGF-β in osteoarthritic bone is seen. However, whether the increase in this growth factor, or other putative anabolic factors such as insulin-like growth factor – 1, platelet derived growth factor, or fibroblast growth factor, precedes the damage to cartilage or is a response to it is unknown.

Estrogen

The perimenopausal increase in the incidence in OA, and the greater overall prevalence of OA in older women suggests an influence of estrogen. In cross-sectional epidemiological studies of hormone replacement therapy and OA, an inverse relationship is commonly seen (30). In the Study of Osteoporotic Fractures (31), ~4,000 Caucasian women 65 or older were studied. 17.2% of women had radiographic evidence of mild to moderate OA of the hip. Risk of OA was significantly reduced in current estrogen users (adjusted odds ratio = 0.62), and current use for more than ten years was associated with a greater reduction in risk than use for less than ten years. In a cohort study of ~1,000 women age 45-64 that compared current users of estrogen replacement therapy to non-users, current users had a decreased risk of OA in the knee (adjusted odds ratio = 0.31) and distal interphalangeal joint (odds ratio = 0.48) (32). The mechanism behind this observation is not clear. Cartilage and bone are hormonally responsive tissues. Chondrocytes in culture have been shown to secrete more of the inflammatory cytokine IL-6 in response to estradiol and IL-1 than to IL-1 alone.(33). In vivo effects have not been demonstrated. As with all retrospective epidemiological studies, there

are methodological concerns about controls and selection bias in the studies of OA and estrogen. It may be that estrogen use is a marker for other factors that protect from OA. Longitudinal studies are underway to determine whether hormone replacement therapy will be useful as a preventative strategy in OA.

Joint Injury

Destabilization of the joint (e.g., by transection of the anterior cruciate ligament) is a common technique to induce OA in experimental animals. It stands to reason that major trauma is also a risk factor for OA in humans. In retrospective studies, a relative risk of >5 for men and >3 for women with major knee trauma has been associated with OA. The typical injuries are cruciate ligament and meniscal tears. A history of such trauma is found in approximately 40% of men and 20% of women with OA of the knee. In one retrospective study, un-repaired cruciate rupture was associated with OA in 90% of cases (34). Radiographic evidence of disease can be seen as early as 2 years after injury (35). In a follow-up of 55 patients (mean age of 28) who had an ACL rupture repaired 9-16 years earlier, 36% had radiographic evidence of OA (36). Up to 50% of patients who also had a torn meniscus had OA by x-ray. In patients with torn menisci only, evidence of OA has been documented in 21% of patients within the first 10 years after injury, compared to 4% in age- and gender-matched controls (37). This rose to 53% in patients and 13% in controls at 30 years after injury.

Occupation and Sports

In the absence of overt joint trauma, repetitive motions that place joints under stress are also associated with the development of OA. In particular, occupations that involve activities such as crawling, crouching, bending, kneeling, or carrying heavy loads during mid-life have nearly double the rate of OA in the knee (38, 39). Prolonged standing, bending, and walking over rough ground have been postulated as motions that predispose farmers to OA of the hip (40). Lastly, OA in the hand has recently been associated with grip strength in men (41), validating earlier studies demonstrating OA in cotton mill workers who use their hands in pinching or twisting motions (42).

In the case of sports, it is difficult to separate the effects of joint trauma such as meniscal or ligament tears from the effects of repeated high-impact loading of the joint. Most studies have looked at former elite athletes. In the absence of joint injury, there is little evidence that recreational sports such as jogging are associated with OA (43). Studies of elite athletes have been conflicting. However, several well-controlled retrospective studies have shown a correlation of high-impact or weight-bearing activities during adolescence and young adulthood and the development of OA in later years. In one study of 117 male former top-level athletes aged 45-68, radiographic OA was seen in 3% of shooters, 29% of soccer players, 31% of weight lifters, and 14% of runners (44). Subjects with radiographic changes were more likely to report clinical symptoms. In a study of 81 female ex-runners and tennis players age 40-65, OA of the knees and hips was 2-3 fold more common than in age- and weight-matched population controls (45). Interestingly, this study also reported increases in radiographic OA among non-athletes who consistently participated in more than one hour of vigorous recreational exercise over a 20-30 year period, suggesting that prolonged repetitive activity is a risk factor for OA.

Prevention of Osteoarthritis

Felson (46) has recently reviewed the epidemiology of OA and commented on various preventative strategies. Obviously, for a problem with a prevalence as great as OA, even small changes in incidence or progression of disease will have great impacts on both the patient, and on societal costs. Primary prevention strategies are aimed at decreasing the incidence of OA in persons without the disease. From the above discussions, persons at high risk for the development of OA, particularly knee or hip involvement, have one or more of the following factors:

- Age over 50
- Female
- First-degree relative with OA
- Obesity
- Major joint injury/surgery
- Job requiring bending, carrying, kneeling, etc

Only the last three factors can be modified. By applying published estimates for the incidence of symptomatic disease and prevalence for each risk factor, it is possible to estimate the potential reduction in disease from each of these factors.

Strategy, group	Knee OA prevented, %	Hip OA prevented, %
Eliminating obesity		
Men	26.6 – 51.8	26.1
Women	27.5 – 52.9	26.9
Preventing knee injury		
Men	25.3	-
Women	13.8	-
Eliminating jobs requiring kneeling, carrying heavy loads, etc.		
Men	15 - 30	

Adapted from (46).

A substantial proportion of primary OA could be prevented by modifying these three risk factors. There are currently no clear ways to implement a secondary prevention strategy for OA. That is, to decrease the progression of the disease from an asymptomatic radiographic finding to symptomatic OA. Tertiary prevention, the decrease in symptoms and disability in patients who already manifest clinical osteoarthritis, is likewise a difficult task. There is preliminary evidence that weight loss decreases discomfort in knee OA and that quadriceps strengthening improves disability in older adults (47). Further longitudinal studies are underway to test these and other strategies (e.g., estrogen) for prevention of OA.

BIOLOGY OF CARTILAGE

Osteoarthritis is a disease of cartilage. The chondrocyte, , the cellular element of cartilage, is at the center of the current models of OA pathogenesis and the design of novel treatments. Before considering mechanisms of cartilage destruction in OA, it is worth noting several aspects of cartilage structure and function.

Cartilage structure

Cartilage is a hydrated gel. Its function is to lubricate the joint and protect the subchondral bone form the forces of impact loading. Despite the fact that it must withstand forces of up to 2,000 pounds per square inch when walking, cartilage is ~70% water. Collagen makes up 15-25% of the wet weight of cartilage and 60% of the dry weight. The density of collagen in cartilage is not uniform, being much higher at the articular surface than in the deep layer near subchondral bone. Several types of collagens are found in mammalian cartilage:

Type II Collagen – The principal collagen of cartilage. It forms fibrils that provide the stiffness and tensile strength of cartilage.
Type IX Collagen – Forms a fibril that covalently attaches to Type II in order to provide lateral stability.
Type XI Collagen – A minor type of collagen that is found mixed with Type II fibrils.
Type X Collagen – A short chain collagen that is only seen in hypertrophic cartilage

Proteins make up ~15% of the dry weight of cartilage and glycosaminoglycans ~25%. The characteristic proteoglycan of cartilage is called **aggrecan**. This structure consists of an extended protein core that has two globular domains, a linear domain to which ~30 keratan sulfate and ~100 chondroitin sulfate chains are attached, and a third globular domain (Figure 3).

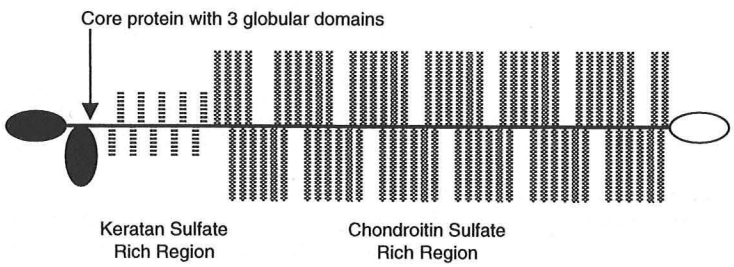

Figure 3 Structure of the Aggrecan monomer

Aggrecan spontaneously associates with long chains of hyaluronic acid that are interspersed within the fibrilar network of collagen (Figure 4), particularly at the deep zones of the cartilage matrix. The high fixed charge density of sulfate and carboxyl groups bind water and cause the matrix to swell. This leads to the compressive stiffness of cartilage.

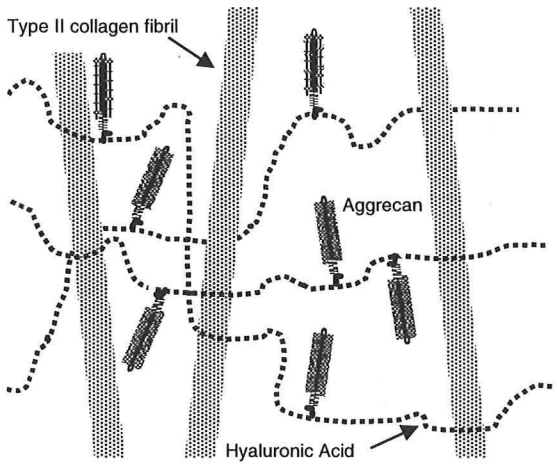

Figure 4 Cartilage Matrix (adapted from (48))

Despite their importance, chondrocytes are relatively rare within the matrix. There are only 10,000 cells per mm^3, located within the deep regions of cartilage. Chondrocytes are responsible for the synthesis of collagen and proteoglycans. Chondrocytes derive from mesenchymal cells. In the adult, they do not normally divide. When stimulated by cytokines, or in response to injury, chondrocytes have a limited ability to divide again, albeit at a slow rate.

Regulation of Matrix Synthesis

The ability of chondrocytes to synthesize collagen and proteoglycan is influenced by mechanical and chemical factors. Both isolated chondrocytes and cartilage in culture require cyclic changes in ambient hydrostatic pressure for optimal function. For example, chick cartilage responds to "pulses" (4 seconds on, 11 seconds off) of physiologic compressive forces by increasing cellular cAMP content and increasing collagen and proteoglycan synthesis. An increase in general protein synthesis is not seen, nor are these effects present when different loading cycles are used or either sub- or supraphysiologic forces are applied.

A number of cytokines and growth factors have effects on chondrocyte proliferation and matrix synthesis. Many of these factors also play a role in the induction of degradative pathways by chondrocytes discussed below.

Factor	Proliferation	Synthesis	
		Aggrecan	Collagen
IGF-1	↑↑	↑↑	↑↑
IGF-II	↑	↑	↑
Insulin	↑	↑	↑
Growth Hormone	↑	↑↑↑	↑↑↑
TGF-β	↑	↑↑↑	↑↑↑
Basic FGF	↑↑↑↑	↑↑↑↑	↑
EGF	none	↑	↑
PDGF	↑↑	↑↑	↑↑
IL-1	↓	↓	↓
TNF-α	↓	↓	↓
γ-Interferon	?	?	↓

Adapted from (48).

Matrix Degradation

Like bone, cartilage undergoes continuous change during growth and development, and regeneration and repair during adult life. The degradation of the matrix necessary to remodel or repair cartilage is achieved mainly by proteases secreted by chondrocytes. Other possible sources of proteases include synoviocytes and polymorphonuclear leukocytes. The primary proteases involved in cartilage degradation are the matrix metalloproteinases (MMP), a family of related 43-85 kDa proteins that are able to degrade both collagen and proteoglycan.

Enzyme	MMP	Cartilage Matrix Substrates
Collagenase-1	MMP-1	Types II and X collagen; Aggrecan
Collagenase-3	MMP-13	Type II collagen; others?
Gelatinase A	MMP-2	Denatured Type II collagen; native Types X and XI collagen
Gelatinase B	MMP-9	Type XI collagen; Aggrecan
Stromelysin-1 and –2	MMP-3 and –10	Types II, IX, X, and XI collagen; Aggrecan

MMPs are synthesized as pro-enzymes that are activated by proteolytic cleavage. Plasmin is probably the major activating protease in cartilage, although once activated, stromelysin is capable of activating pro-collagenases. All the MMPs contain one atom of zinc at their active site, which is crucial for their function and a target for therapy (see below). The synthesis of MMP is regulated by cytokines and growth factors. Both IL-1 and TNF-α promote MMP synthesis while glucocorticoids and TGF-β inhibit the production of MMP. Gamma interferon both inhibits matrix synthesis as well as the synthesis of degradative enzymes.

The activity of metalloproteinases is counterbalanced by naturally occurring 21-28 kDa proteins termed tissue inhibitors of metalloproteinases (TIMP-1, 2, 3, 4). These proteins

are capable of inhibiting collagenases, gelatinases and stromelysins. In general, the synthesis of these inhibitors in OA cartilage explants is up-regulated by TGF-β (49), and decreased by IL-1 (50). It is thought that in established OA, the level of TIMP is not sufficient to inhibit the increased amount of MMP (51). Whereas in normal cartilage there is a small excess of inhibitor to protease concentration, in OA cartilage the concentration of proteases increases three-fold greater than the TIMP concentration. The balance between MMPs and TIMPs is clearly complex as both increased (49) and decreased (52) expression of TIMP-1 mRNA has been seen in OA cartilage compared to non-OA cartilage. There may also be differences in the expression of TIMP-1 and TIMP-2 that are important for disease progression. TIMP-1 appears to be inducible by TGF-β, while TIMP-2 is constitutively expressed, suggesting a role of TIMP-2 in normal cartilage homeostasis and TIMP-1 in repair (49).

Factor	MMP Synthesis	TIMP Synthesis
IL-1	↑	↓
TNF-α	↑	?
TGF-β	↓	↑
Glucocorticoids	↓	no effect

CARTILAGE PHYSIOLOGY IN OSTEOARTHRITIS – SUMMARY

Taken together, these features of cartilage physiology can be used to explain the disease process in osteoarthritis:

- Damage to the collagen network caused by mechanical factors, reactive oxygen intermediates, or non-enzymatic glycosylation (53).
- Swelling of proteoglycans with increased water content.
- Increased in IL-1 produced by chondrocytes, fibroblasts, or synovial cells. Increase in TNF-α by chondrocytes, PMN, or monocytes. Spontaneous production of nitric oxide by chondrocytes.
- Initial upregulation of new collagen and proteoglycan synthesis.
- Increase in matrix metalloproteinase content leading to eventual decrease in collagen and glycosaminoglycans in cartilage.
- Decrease in proteinase inhibitor content.
- Fracture of cartilage matrix beginning at the articular surface.
- Loss of cartilage volume.

NEW STRATEGIES IN THE MANAGEMENT OF OSTEOARTHRITIS

Over the past 5-10 years, several new therapeutic paradigms have emerged for the treatment of OA. With the understanding that OA truly is an inflammatory condition, it has been possible to design therapies that inhibit particular cytokines or counteract degradative enzymes. These therapies are still being tested *in vitro*, or in animal models of OA. Two new therapies have recently been approved by the Food and Drug Administration for treatment of OA. The first is intra-articular hyaluronic acid, an agent designed to improve the mechanical properties of the joint, but which may also have immunomodulatory effects. The second pharmaceutical is the first of a class of

selective cyclooxygenase-2 (COX-2) inhibitors designed to treat pain in OA without the gastrointestinal side effects seen with traditional non-steroidal anti-inflammatory agents (NSAIDS).

- Role of nitric oxide in OA
- Inhibition of metalloproteinases as a therapeutic target
- Intra-articular hyaluronic acid
- COX-2 inhibition

Role of Nitric Oxide in Osteoarthritis

Nitric oxide (NO) is a highly reactive free radical produced by the oxidation of L-arginine. Three isoforms of nitric oxide synthase (NOS) are responsible for the production of NO. NOS-I and NOS-III are constitutively expressed enzymes in neuronal cells and endothelium, respectively (54). Production of NO at these sites regulates neuronal transmission and vascular smooth muscle tone. The third NOS isoform, NOS-II is expressed in an inducible manner by a number of cell types exposed to inflammatory mediators such as IL-1, TNF-α, and lipopolysaccharide (LPS). Once expressed, this form of NOS produces large amounts of NO over sustained periods of time. NO rapidly reacts with oxygen and thiol-containing compounds (55). In aerobic media, it quickly forms nitrite and nitrate which can be measured as surrogates for NO production. It reacts with superoxide to form peroxynitrite, a highly cytotoxic compound, contributing to the microbiocidal effect of NO. Lastly, it modifies the function of protein within cells by nitrosylation of critical amino acids such as tyrosine or cysteine.

Increased concentrations of nitrites and nitrosothiols have been documented in synovial fluids of patients with both rheumatoid and osteoarthritis, suggesting a role for NO in the pathogenesis of OA (56). Moreover, several groups have directly identified the expression of NOS-II mRNA or protein in both synovial cells and chondrocytes in OA (57). A recent immunohistochemical study looked at the expression of NOS-II, IL-1, and TNF by synovium and cartilage in a series of patients with RA, OA and traumatic arthritis (58). Whereas all three proteins were highly expressed in RA synovium, they were found less frequently in RA cartilage. Conversely, there was low expression in OA synovial membrane, but high level expression in OA cartilage. The cells that expressed NOS and TNF were chondrocytes located in the superficial zone that was depleted of glycosaminoglycans. This suggests that the production of NO is restricted to that region of the cartilage matrix that is undergoing degeneration. Patients with traumatic arthritis had findings similar to those with OA. The authors concluded that the production of NO as well as IL-1 and TNF in OA was not driven by the synovium as it is in RA, but that other factors (such as repetitive microtrauma) lead to expression of these proteins by chondrocytes.

Normal cartilage does not make NO unless simulated by IL-1, TNF, or LPS. Cartilage explants from patients with OA undergoing joint replacement spontaneously express NOS and produce NO for up to three days (59). This exceeds the half-life of NOS mRNA, suggesting that there are factors in OA cartilage that stimulate the chondrocyte to continue NO production. These factors could be produced by the chondrocyte itself,

produced by other cell types in the cartilage, or due to interactions between the matrix and the chondrocyte.

Nitric oxide produced by chondrocytes regulates the expression or function of TGF-β and PGE_2. In normal rabbit articular cartilage, IL-1 drives the coordinate expression of both TGF-β and NOS mRNA (60). However, the rapid production of NO represses the expression of TGF-β, and ultimately, proteoglycan synthesis. Inhibition of NOS by the compound L-NMA decreased the amount of nitrite produced, and increased the amount of TGF-β and proteoglycan seen in response to IL-1. The authors suggested that NO production serves normally to limit proliferative responses to TGF-β. In OA, this negative control is excessive and prevents repair.

OA cartilage also spontaneously produces PGE_2 at levels 50-times greater than normal cartilage, owing to the upregulation of COX-2 in chondrocytes (61). The expression of PGE_2 and NO is coordinately regulated in these cells at the level of transcription. Glucocorticoids inhibit production of PGE_2 but not NO. Inhibition of NOS-II resulted in a doubling of the PGE_2 produced and exogenous NO decreased prostaglandin production. This may be a cartilage-specific phenomenon, as NO has been shown to increase prostaglandin synthesis in other models of inflammation. The effect of PGE_2 in OA is not clear. It may contribute to joint inflammation and pain, but may also have a protective effect. In a rodent chondrocyte cell line, PGE_2 stimulates proliferation and aggrecan synthesis (62). In human OA cartilage, it up-regulates glucocorticoid receptors (63). If these observations reflect a protective effect of prostaglandins on cartilage, then further investigation is warranted to determine if analgesic therapies that decrease PGE_2 actually accelerate disease.

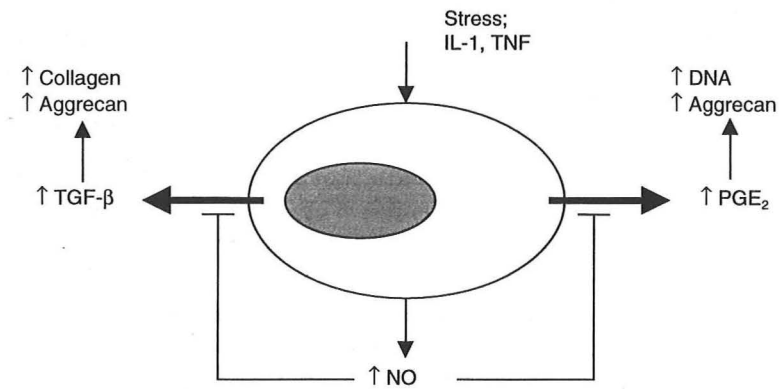

Figure 5. Putative relationship between nitric oxide and PGE_2 and TGF-β

These *in vitro* studies suggest that inhibition of NOS-II would be beneficial in OA. This was tested by Pelletier, *et al.* in the cruciate-deficient dog model of OA (64). In this model, the anterior cruciate ligament of one knee is surgically sectioned in anesthetized animals. The joint instability results in rapid (10 week) progression to a cartilage lesion that is grossly, histologically, and biochemically representative of human OA. In this

study, one group of dogs received no therapy after operation and the other received a selective NOS-II inhibitor, *N*-iminoethyl-L-lysine (L-NIL) at doses previously determined to cause maximal inhibition of NO production in cartilage. A third group was not operated on.

The un-operated dogs had no macroscopic or microscopic cartilage lesions. In the dogs treated with L-NIL, the mean area of cartilage erosion decreased from 15.33 mm^2 to 7.33 mm^2 on the femoral side of the knee, and from 17.02 mm^2 to 9.75 mm^2 on the tibial plateau. Microscopically, the cartilage from L-NIL treated dogs had more glycosaminoglycan and less fibrillation and fissures than the OA controls. Collagenase activity in the cartilage of OA dogs was approximately 3 times that of normal dogs. This was decreased almost to control levels with L-NIL treatment. Inflammatory mediators were measured in synovial fluid.

Mean Synovial Fluid Values:

Factor	Un-operated	OA Control	OA L-NIL
Synovial fluid vol.	0.3 ml	6.8 ml	2.4 ml
IL-1β	0.1 pg	32.9 pg	0.5 pg
Nitrite/nitrate	6.5 nmol	96.4 nmol	48.6 nmol
PGE_2	ND	9.4 ng	0.8 ng

These data suggest that ~50% inhibition of nitric oxide production by a selective NOS-II inhibitor results in marked reduction in both morphologic and biochemical evidence of pathology in an acute surgical model of OA. While this is an extremely interesting finding, it is not known whether similar inhibition of NOS-II can be achieved in humans, whether this will be a safe, and when and for how long NO inhibition must be carried out.

Matrix Metalloproteinases

As described above, the degradation of cartilage matrix is effected by a series of matrix metalloproteinases (MMP) naturally inhibited by TIMPs. Therefore, it is theoretically possible to slow the progression of osteoarthritis by modulating the activity of MMP and TIMP:

- Inhibit the synthesis or release of MMPs
- Inhibit the activation of pro-MMPs
- Inhibit the activity of mature MMPs
- Stimulate the synthesis or activity of TIMPs

While MMP play a role in normal remodeling processes, they are involved in several other pathologic conditions other than OA. These include periodontal disease, cancer metastasis, angiogenesis and corneal ulcers (65). Thus, there is considerable interest in the development of compounds that will inhibit MMP under pathologic conditions, but not affect physiologic MMP functions. The *in vitro* and *in vivo* studies to date have

focused on three types of compounds, tetracyclines, hydroxamic acid derivatives, and specific low molecular weight inhibitors of collagenase.

Tetracyclines inhibit MMP primarily by chelating the zinc atom abolishing activity in the mature enzymes. There is also evidence that prevent activation of pro-MMP and increase the production of TIMP. A series of ten chemically modified tetracyclines (CMT) has been developed which lacks antimicrobial activity but retains the ability to inhibit MMP. In chick and bovine cartilage organ cultures, these agents have been shown to be extremely potent inhibitors of collagenase activity, gelatinase release into the culture medium, and degradation of proteoglycan. The therapeutic potential of tetracyclines *in vivo* has been studied primarily in periodontal disease. Both tetracyclines and CMT inhibit alveolar bone loss in rodent models of gingivitis. A doxycycline derivative, Periostat (CollaGenex) was approved by the FDA in September, 1998, as an adjunct to the treatment of adult periodontitis.

The ability of doxycycline to both prevent and treat OA has been tested in cruciate-deficient dogs (66). In animals that received doxycycline for two months following anterior cruciate ligament transection, there was a 47% decrease in collagenase and 82% decrease in gelatinase activity. Gross and histologic appearance of the OA cartilage was also improved in the doxycycline-treated dogs. Lastly, several short-term studies have been done looking at the effect of tetracycline administration prior to joint replacement for either RA or OA (67, 68). In each case a 50-60% decrease in collagenase activity was seen treated patients compared to untreated controls.

Hydroxamic Acids and Other MMP Inhibitors These include Batimistat and Marimastat, two agents that have been tested in clinical trials of metastatic cancer (69), as well as a number of other orally available agents. *In vitro*, these compounds block release of proteoglycans from IL-1 treated cartilage, with no change in cartilage morphology or chondrocyte viability (70-72). An additional effect of these agents is the prevention of proteolytic release of TNF-α from the cell surface, decreasing the local concentration of this inflammatory cytokine (73-75) Selectivity for osteoarthritis can be demonstrated. Collagenase-3 (MMP-13) is produced normally by chondrocytes (76) and is structurally different from fibroblast collagenase (MMP-1) or PMN collagenase (MMP-8). MMP-13 degrades type II collagen ten times faster than other collagenases (77). A specific inhibitor of MMP-13, RS 102,481, completely blocked the spontaneous degradation of collagen in cartilage explants from patients with OA. Again, there was no effect on either total collagen synthesis or viability.

Human studies on collagenase inhibitors for OA are underway. However, a recent report documented the success of RO 32-3555 (Trocade), an orally active collagenase selective MMP inhibitor in a mouse model of OA (78). The STR/ORT mouse spontaneously develops calcification of the quadriceps tendon and osteophyte formation accompanied by histologic changes in cartilage similar to human OA. Treatment of mice with 50 mg/kg/day of drug for 12 weeks led to the prevention of (and perhaps resolution of) radiographic changes and a 60% decrease in histologic OA.

Intra-Articular Hyaluronic Acid

Hyaluronic acid is a linear glycosaminoglycan consisting of repeating glucuronic acid/N-acetyl glucosamine disaccharides. It is a normal constituent of both synovial fluid and the articular layer of cartilage. In normal synovial fluid, it has a molecular weight of ~10^7 daltons, but in OA and RA the average size can decrease to 2 x 10^5 daltons, due to the activity of degradative enzymes. This results in the loss of lubrication and shock-absorbing properties of synovial fluid.

Two hyaluronic acid products have been approved by the US Food and Drug Administration for use in OA of the knee. Hyalgan (Sanofi) is a linear hyaluronic acid with a mean size of 5 x 10^5 daltons; Synvisc (Wyeth-Ayerst) is a cross-linked hyaluronan. Both products require repeated weekly injections (Hyalgan – five; Synvisc – three), and are quite expensive (approximately $600/series of injections). In addition to their effects as "viscosupplements", there is speculation that these hyaluronic acid derivatives may be immunomodulatory. They bind to cell surfaces through a member of the CD44 adhesion receptor family. Exogenous hyaluronic acid has been shown to reduce leukocyte chemotaxis and activation, as well as prostaglandin and cAMP production.

Clinical trials of intra-articular hyaluronic acid have been difficult to evaluate due to methodological problems with study design and inclusion criteria. The results of several recent studies are summarized below.

Study	Design	# Patients	Outcome
Adams, 1995 (79)	Single-blind	102	Patients receiving Synvisc alone or Synvisc plus NSAID had less pain than those on NSAID alone.
Lussier, 1996 (80)	Open-label	336	87% of patients receiving Synvisc had improved activity levels after two courses, 8 months apart.
Lohmander, 1996 (81)	Double-blind	240	Hyaluronic acid no better than placebo except in group older than 60 and with severe disease
Frizziero, 1998 (82)	Open	40	Biopsies of synovial membrane and cartilage at 6 months showed improvement in 1/3 of patients treated with Hyalgan
Wobig, 1998 (83)	Double-blind	60	39% of patients receiving Synvisc were pain-free 10-24 weeks post-injection compared to 13% of placebo.

Adverse events with hyaluronic acid injection appear to be limited to transient pain and swelling of the injected knee seen in 5-10% of patients (per series of injections). Although a formal meta-analysis of different studies has not been done, it appears fair to conclude that intra-articular viscosupplementation with hyaluronic acid is safe, and may provide pain relief to a sub-set of patients. Due to the high placebo response seen

in all controlled studies, a large double-blind comparison will be needed to accurately define those patients who will benefit the most. The major drawbacks of this therapy are the need for repeated office visits and cost.

Specific COX-2 Inhibition

Treatment of pain and its attendant decrease in function are major goals in OA therapy. The most common class of medications for this are non-steroidal anti-inflammatory drugs (NSAIDS). It is estimated that with over 80 million NSAID prescriptions written each year and increasing over the counter use, nearly one in seven Americans is taking an NSAID. Both short- and long-term studies have shown that NSAIDs are better than placebo in the treatment of pain from OA (84). However, NSAIDs have significant toxicity, particularly in the elderly – the population with the greatest prevalence of OA. These toxicities include gastric erosion and ulceration, altered renal physiology, and inhibition of platelet function. The scope of these side effects, particularly the GI toxicity (see below) has prompted several groups, including the American College of Rheumatology (85, 86), to recommend other analgesics such as acetaminophen for the initial treatment of OA. These conclusions are based on three studies showing that up to 4 grams of acetaminophen per day was better than placebo and no different than NSAIDs in the treatment of knee pain from OA (87, 88). In the longest of these studies, 2,600 mg/d of acetaminophen was compared to 750 mg/d of naproxen in 187 patients treated for two years. The only significant difference in outcome was more relief from pain at rest in the naproxen group. However, approximately two-thirds of each group dropped out. More of the dropout in the acetaminophen group was for lack of efficacy, and more of the dropout from the NSAID group was from adverse effect. Lane has summarized the clinical experience of most rheumatologists who find that acetaminophen can be effective for initial treatment of mild to moderate pain, but that many patients will end up on NSAIDs as they request more potent analgesics. Clearly, the side effect profile of traditional NSAIDs has prevented the rigorous comparison of these agents to acetaminophen.

The most common serious side effect of NSAIDs is NSAID gastropathy. This can include asymptomatic mucosal damage seen endoscopically, symptoms of pain, dyspepsia, and reflux, and frank bleeding or ulceration. Unfortunately, there is little correlation among these three events. Up to 80% of patients taking NSAIDs have endoscopic erosions, depending of their definition, although only 1-2% have serious GI complications (89, 90). Dyspepsia occurs in 10-12% of patients on average, but may range up to 50% depending on the drug. Most worrisome is the fact that there is no clear association between symptoms and serious complications such as ulcers or GI perforation. 50-60% of patients with serious NSAID-related GI complications (perforation, bleeding, or death) were asymptomatic (91).

The prevalence of gastroduodenal ulcers in patients taking NSAIDs ranges from 15-22%. McCarthy has presented data from an endoscopic study where 13% of NSAID users had gastric ulcers and 11% had duodenal ulcers as compared to 0.28% and 1.4% in the control population (92). This has been put into perspective for patients with RA and OA using the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) database. This is a multi-center, prospective data bank on clinical and out-

come information on 36,000 patients in the US and Canada, including drug use and side effects (91).

	OA Hospitalizations	RA Hospitalizations	RA Deaths
Number of patients	1,283	2,921	2,921
Person-years	3,234	12,224	12,224
Rate (%) per year on NSAID	0.73 ± 0.18	1.46 ± 0.13	0.22
Rate (%) per year not on NSAID	0.29	0.27	0.05
Relative risk	2.51	5.49	4.21

The authors conservatively estimated that the total number of hospitalizations from NSAID-related GI complications in the US was 107,000 (51,000 RA and 56,000 OA) with 16,500 NSAID-related deaths (7,700 RA and 8,800 OA). These are conservative figures based on only 8 million persons with OA taking NSAIDs. The annual cost of these complications was estimated to be in excess of $1 billion in medical costs alone.

Several studies have outlined the risks for NSAID gastropathy. They include older age, history of GI bleeding or ulcer, steroid use, and anticoagulant use (93-96).

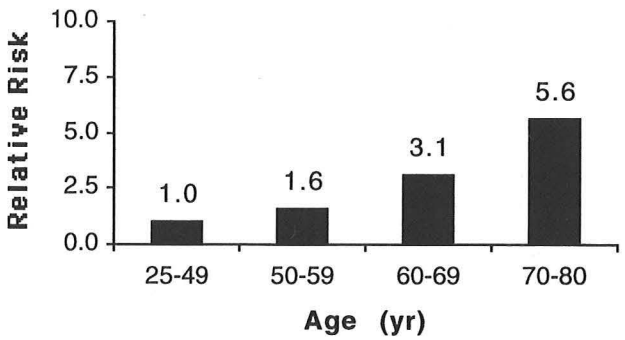

Figure 6. Risk of GI bleeding or ulcer by age (91)

Factor	Relative Risk/ Odds Ratio
History of previous peptic ulcer	9.5
History of previous GI bleed	6.7
Concomitant use of corticosteroids	4.4
Concomitant use of anticoagulants	12-16
NSAID dose >120% average	7.7
Concomitant use of ≥ NSAIDs	23.3
Regular use of NSAID plus aspirin	3.6

Obviously, these risk factors coincide with the epidemiology of OA, thus putting these patients at the highest risk for the development of NSAID side effects.

The therapeutic and toxic effects of NSAIDs both derive from the inhibition of prostaglandin synthesis by the enzyme cyclooxygenase (COX). This enzyme contains both a cyclooxygenase and endoperoxidase activity that converts arachidonic acid to PGH_2. There are two isoforms of COX, COX-1 and COX-2. The catalytic functions of these two enzymes are quite similar as their core protein sequences are 75% identical. The critical differences between COX-1 and COX-2 are in their pattern of expression (97).

	COX-1	**COX-2**
Critical gene promoter elements	"TATA-less" – a feature of non-inducible housekeeping genes	TATA box, multiple Nf-κB sites and cAMP response element
mRNA features	Stable	Long 3'-untranslated region confers rapid degradation
Pattern of expression	All tissues except RBC, including GI tract, platelets, renal medulla, endothelium, **and both RA and OA synovium**.	Stimulated mononuclear cells, synovial cells, endothelial cells, chondrocytes; unstimulated neocortex, hippocampus, female reproductive tract, macula densa of kidney (rat) and colon cancers
Factors that increase expression		IL-1, TNF-α, LPS, TGF-β, EGF, PGDF, FGF
Factors that decease expression		Glucocorticoids, IL-4, IL-13

The highly regulated expression of COX-2 in areas of inflammation suggests that it is responsible for most, if not all the pathologic production of prostaglandin in response to tissue injury. This has been verified in animal models where selective COX-2, but not selective COX-1 inhibitors are able to prevent pain and tissue edema. COX-1 is thought of as a "housekeeping" enzyme, producing protective prostaglandins in gastric mucosa, the kidney endothelial cells, and platelets.

The clear-cut role of COX-1 as a protective enzyme and COX-2 as pathologic is altered somewhat, though, by analysis of mice carrying a targeted disruption of either gene. Surprisingly, COX-1 knockout mice had little baseline or indomethacin-induced gastric pathology, indicating the presence of unknown compensatory factors(98). Prostaglandin-dependent inflammatory responses were intact. While female mice became pregnant in the absence of COX-1, fetal wastage approached 90%.

COX-2 knockout mice lacked LPS-induced PGE_2 production, as expected (99). Surprisingly, a large number of mice died at ~ 8 weeks of age. These mice consistently had small kidneys with hypoplastic nephrons and atrophic glomeruli. Renal histology was normal at birth, suggesting a requisite role for COX-2 in post-natal renal development. Finally, female COX-2 deficient mice have defective ovulation, and a complete failure of fertilization and blastocyst implantation (100). This effect could be mimicked in wild-type mice by a specific inhibitor of COX-2, resulting in a 50% decrease in fertility.

The three-dimensional structures of human COX-1 and COX-2 have been determined (reviewed in (101)). They are both dimers with three independent domains. They bind to one leaflet of a lipid bilayer, allowing access to arachidonic acid released by phospholipase A2. The active sites for both enzymes is contained within a long hydrophobic channel. A conserved arginine within the channel serves the binding site for traditional NSAIDs an blocks access of substrate to the active site. The major difference between the two isoforms is the presence of a large "side pocket" along the channel of COX-2. This pocket can accommodate a large hydrophilic group on specific COX-2 inhibitors (usually a sulfonamide, sulfonyl, or sulfone) which prevents access of these compounds into the active site of COX-1.

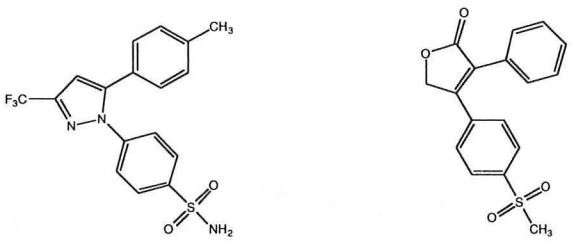

Figure 7 Structure of specific COX-2 inhibitors celecoxib (left) and rofecoxib (right)

Individual NSAIDs have different potencies for the inhibition of COX-1 and COX-2. The actual values depend on whether human or rodent enzymes are being tested, and whether assays are done on recombinant proteins, or whole cells or tissues. In a study by Cryer and Feldman at the Dallas VAMC, blood and gastric biopsies were obtained from normal volunteers (102). The IC_{50} values were determined for 25 different NSAIDs on COX-1 and COX-2 in blood and total COX in gastric mucosa. The values differed over 4 orders of magnitude for the different compounds. The relative selectivity of certain NSAIDs can be determined by the ratio of the IC_{50} of COX-2 to that of COX-1 in the blood. Cryer and Feldman compared this value to the relative potency of the different agents to inhibit gastric prostaglandins. In the table below this is compared to a ranking of GI toxicity taken from the ARAMIS database (91):

Drug	Ratio of IC_{50} (Blood)	Gastric Mucosa (Rank)	Relative GI Toxicity
Fluribiprofen (Ansaid)	10.27	3	
Ketoprofen (Orudis)	8.16	1	1
Oxaprosin (Daypro)	2.52	8	
Tolmetin (Tolectin)	2.09	10	4
Indomethacin (Indocin)	1.78	6	2
Ibuprofen (Motrin)	1.69	5	7
Naproxen (Naprosyn)	0.88	4	5
Piroxicam (Feldene)	0.79	7	3
Nabumetone (Relafen)	0.62	11	
Etodolac (Lodine)	0.12	9	
Diclofenac (Voltaren)	0.05	2	6

From these data it can be seen that there is great variation in the COX-2:COX-1 selectivity of traditional NSAIDs. The relative ability of a given drug to preferentially inhibit COX-2 in blood was only loosely related to its ability to inhibit gastric prostaglandin production. Moreover, the IC_{50} for gastric prostaglandin synthesis for all agents tested was lower than the mean serum concentration normally achieved *in vivo*. Finally, the GI toxicity of a drug is related not only to its ability to inhibit gastric prostaglandins, but also to half-life, pKa, etc.

Most large pharmaceutical companies have designed drugs that specifically inhibit COX-2 with little or no COX-1 inhibition. Celecoxib (Celebrex™, Searle/Pfizer) has been launched earlier this year, and rofecoxib (Vioxx™, Merck) is to be reviewed by the FDA in April, 1999. The IC_{50} ratio for celecoxib using recombinant enzymes is 0.0026 and for rofecoxib it is 0.0012 (103). Both agents have been shown to lack inhibition of gastric prostaglandin synthesis (104, 105).

In clinical studies reported to date (most in abstract form), both celecoxib and rofecoxib have used by several thousand patients for up to a year or longer. They have been shown to have efficacy in RA and OA that is better than placebo and similar to traditional NSAIDs (106-108).

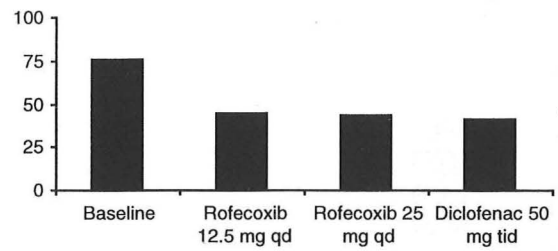

Figure 8 Results of a double-blind, multi-center trial of rofecoxib vs diclofenac in OA of the hip or knee in 784 patients [Cannon, 1998 #2006]. Results of pain while walking on a flat surface (0-100 scale) are shown.

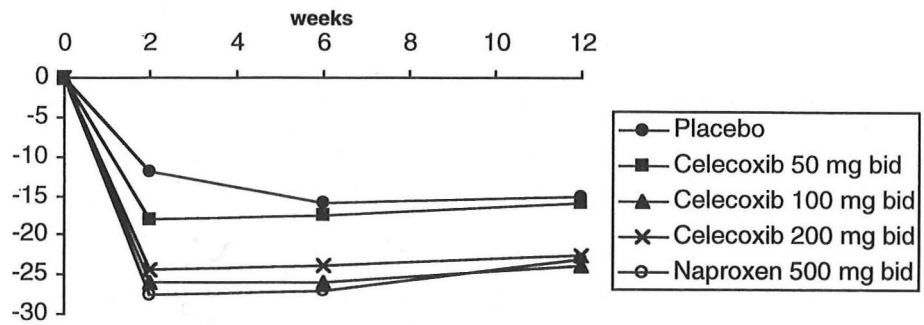

Figure 9 Results of a double-blind trial of placebo, celecoxib, and naproxen in 998 patients with a flare of OA of the knee. Decrease in pain (0-100 scale) is shown.

A low rate of incident GI bleeding has been seen with these agents. Two of 5,285 patients (0.04%) receiving maximum doses of celecoxib developed significant GI bleeding compared to historical values of ~1% for non-selective NSAIDs. Endoscopic studies have documented ulcer rates similar to background for COX-2 specific agents, while traditional NSAIDs had gastroduodenal ulcer rates of up to 16% in 3 months. Erosions were seen in nearly all patients taking traditional NSAIDs while patients on COX-2 inhibitors had rates similar to placebo.

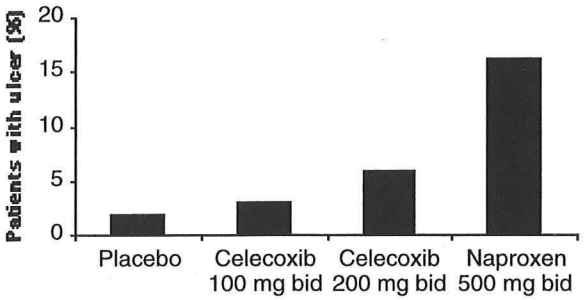

Figure 10. Gastroduodenal ulcer prevalence in patients treated with celecoxib vs naproxen. Data from Searle Phase III Clinical Studies. Similar data are seen in a published short-term trial of naproxen and celecoxib (109)[Simon, 1998 #2023].

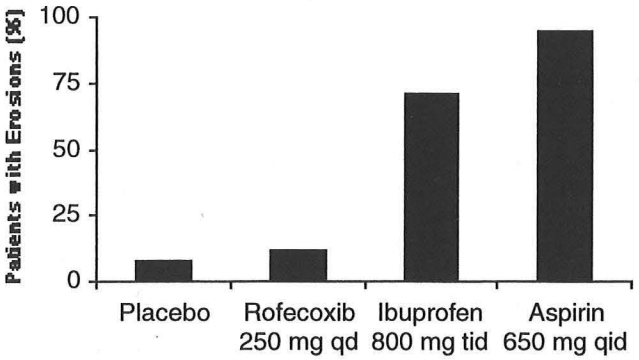

Figure 11 Prevalence of gastroduodenal erosions in patients treated with rofecoxib (at 10x standard dose), ibuprofen, or aspirin for 7 days (110)[Lanza, 1997 #2025].

Data used in the FDA approval of celecoxib suggests that the rate of dyspepsia (8.8%) was intermediate between placebo (2.8%) and traditional NSAIDs (7.9 – 9.0%), although rates of discontinuance for GI side effects were similar to placebo. Despite these results the studies for celecoxib were not of sufficient power to discriminate between it and placebo. Also the relationship between endoscopic findings and clinically significant events is not certain. Therefore, the FDA has required celecoxib to retain the standard warning for GI side effects, pending post-marketing data. Whether rofecoxib and other COX-2 inhibitors will also have this requirement is not known.

Platelet function *in vitro* and bleeding time are unaffected by either agent, as predicted by their lack of COX-1 effect. Effects on renal function in patients dependent on renal prostaglandin synthesis are unknown.

Specific COX-2 inhibitors – Recommendations and Concerns

It appears that celecoxib and rofecoxib, the two specific COX-2 inhibitors with the most clinical data, have efficacy similar to non-selective NSAIDs, without significant GI toxicity. As greater familiarity with these and other COX-2 inhibitors is gained, they are likely to become part of the first-line treatment for OA, RA, and many other causes of acute and chronic pain. They should certainly be considered in older patients and any patient with other risk factors for GI side effects of NSAIDs. The size of the NSAID market makes this currently an extremely competitive area. Second-generation agents with different potencies and pharmacokinetics are being developed. Studies are underway to test these agents directly against acetaminophen.

As with all new classes of pharmaceuticals, there are important questions to be answered about the specific COX-2 inhibitors.

- What is the role of COX-1 in human painful/inflammatory conditions?
- What is the effect of COX-2 inhibitors in patients with GI inflammation – active *H. pylori* gastritis, healing ulcers, or inflammatory bowel disease?
- What is the effect on renal function? In clinical studies, the rate of peripheral edema was higher in patients receiving COX-2 inhibitors than in placebo, perhaps reflecting the expression of COX-2 in the macula densa.
- What is the role of COX-2 in maintaining endothelial prostacyclin production? Will patients taking aspirin for cardiovascular prevention still benefit from COX-2 specific inhibition?
- Can they be used safely in patients taking oral anticoagulants or otherwise at additional risk for upper GI bleeding – e.g., the presence of portal hypertension.?

CONCLUSIONS

- Osteoarthritis exacts a large toll on a significant fraction of the population, particularly among the elderly.
- It is a major cause of morbidity and medical costs.
- There are inflammatory features to osteoarthritis, although at a lower level than seen in rheumatoid arthritis.
- Inhibition of these inflammatory features through agents designed to block cytokine action, nitric oxide production, or metalloproteinase activity holds the promise to be a "disease-modifying" strategy for osteoarthritis.
- New therapies such as intra-articular hyaluronic acid and specific COX-2 inhibitors promise pain relief with elimination of side-effects.

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