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Animal Models of Arthritis: Pharmacological Intervention

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Rheumatoid arthritis is an incurable chronic inflammatory and destructive arthopathy that affects 1% of the population world-wide. It has substantial personal, social and economic costs. The long-term prognosis is poor: 80 percent of affected patients will become disabled within 20 years after onset of disease. Medical costs of rheumatoid arthritis average ~$ 6000 (US) per patient (1). Current antirheumatic drugs have limited efficacy and many side effects and more importantly they do not improve the long-term prognosis of rheumatoid arthritis (2). After a decade of few notable advances in therapy, several biological response modifiers that target pathophysiological processes in the disease have now emerged in the clinic. These new drugs are termed biological agents, and although information about their use in the clinic is still limited to short term treatment, they appear to have the ability to modify disease progress. In addition, COX-2 selective agents have now been approved that have comparable efficacy with standard NSAIDs, but fewer gastrointestinal side effects (3). Thus today many more therapeutic options are suddenly open to patients that even five years ago had little hope of relief from chronic pain and inflammation.

These impressive advances are the result of many years of work in determining the mechanisms involved in inflammation and pain of arthritis. New insights into the cytokines involved in inflammation and the effects they have on various inflammatory cells, as well as the identification of a second COX isozyme, COX-2, which appears to be specific for inflammatory processes have helped significantly in developing these new strategies for therapeutic intervention. This summary will briefly review cytokine targeted strategies (focusing on TNF-α and IL-1) and also the development of COX-2 selective agents.

Cytokines and Rheumatoid arthritis

Though many details remain unclear, most notably the initiator of this process, a clearer picture of the pathophysiology of rheumatoid arthritis is beginning to emerge. Initiation of this perpetual inflammatory condition is thought to be due to the presentation of a still unknown antigen that stimulates T cells (4). This probably occurs in genetically susceptible individuals and is characteristic of the early stage of disease. The activated T cells in the

synovium produce a variety of cytokines, which include interferon-γ (IFN-γ), interleukin (IL) -2, IL-6 and tumour necrosis factor-α (TNFα). These cytokines conserve and perpetuate the inflammatory status of the synovium. Other cell types also become stimulated in the synovium (B cells, monocytes, dendritic cells, fibroblast-like synoviocytes) either by cytokines or by direct interactions with activated T cells. This results in a second and more destructive stage of the disease (1). Activated monocytes and fibroblastic synoviocytes produce a variety of proinflammatory cytokines such as IL-1 and TNFα as well as growth factors. In addition, they stimulate the production of matrix metalloproteinases and other proteases that mediate the breakdown of tissue matrix of the joint that characterises the destructive phase of rheumatoid arthritis (1).

In a chronic inflammatory environment in the synovium, it appears that the upregulation of multiple endogenous anti-inflammatory mediators is not sufficient to suppress this synovitis. Abundant expression of IL-10 and TGFβ has been detected in synovial fluid of arthritic patients, as has elevated levels of the soluble forms of the TNF receptor (p55 and p75 TNFR). These soluble forms of the receptor are natural inhibitors of TNF, however, appear to be present in concentrations that are not able to neutralise TNFα. Similarly, an endogenous inhibitor of IL-1, IL-1 receptor antagonist, or IL-1ra, has also been measured in synovial fluid and is probably released by neutrophils (1,5). However, it is believed that a large excess of IL-1ra is required before it can induce an inhibitory effect. Thus there is an imbalance in the synovium between proinflammatory and anti-inflammatory cytokines. This was the basis of the hypothesis that alteration of this cytokine environment may result in effective therapy in rheumatoid arthritis.

Animal Models of Intervention: TNFα and IL-1

The extensive presence of such a large number of cytokines in the synovium of patients with rheumatoid arthritis, regardless of disease duration, severity or drug therapy implies that rheumatoid arthritis is associated with a prolonged expression of cytokines, rather than a short-lived, acute release. In addition, with so many redundant systems, it was considered initially of little value to inhibit only one cytokine. It was thought that cytokines were all independently regulated, rather than in some co-ordinated manner (6). However, the contrary was shown with a key observation in the early 1990's (7) using animal models of arthritis that indicated that TNFα was an important regulator of other cytokines and could modulate the proinflammatory response.

Williams et al (7) used a specific anti-TNF monoclonal antibody in a murine model of collagen-induced arthritis and measured not only a reduction in disease onset, but also in the

severity of established disease. In this animal model collagen type II emulsified in incomplete Freund's adjuvant is injected intradermally in genetically susceptible animal strains. This model is unique among animal models of arthritis since disease induction occurs by an autoimmune response to normal cartilage protein rather than to an exogenous foreign antigen. Polyarthritis develops within 2-4 weeks in 90% of animals after collagen type II immunization. Erosions due to pannus formation and angiogenesis are observed and joint destruction can be observed after 2 weeks of arthritis onset. Also characteristic is the development of antibodies to cartilage type II. This arthritic model also appears to be T lymphocyte specific since athymic animals are resistant to disease and administration of antibodies to CD4+ T lymphocytes can the inhibit the onset of disease (9).

Injection of an anti-TNF monoclonal antibody reduced joint inflammation and destruction, not only when given before induction of arthritis, but also therapeutically, when given after disease onset (7, 10). Wooley et al (10) demonstrated that incidence of disease in collagen-induced arthritis in DBA/1 mice was reduced by 86% as compared to controls (reduced 28%), when they were injected with 25 μg i.p. of the TNFR:Fc protein daily for one week, beginning 3 weeks post immunization. The severity of disease in the mice with disease was also significantly reduced based both on arthritis index and joint count. In the treatment of arthritis, the same dose was given daily for 2 weeks, beginning at disease onset. TNFR:Fc reduced the severity of arthritis significantly 7.5 and 10 weeks after disease onset.

There is now a huge surge in products that inhibit TNFα, the initial work with the TNF antibody described above has resulted in the marketed drug, Infliximab (Centocor, Remicade®), which is a chimeric anti-TNFα antibody approved for clinical use (11, 12). Two TNF-R Fc fusion proteins have been produced by molecular engineering techniques. These take advantage of the endogenous inhibitory mechanisms of the soluble TNF receptors. Lenercept, a double p55 soluble TNF-receptor construct (Roche) failed to demonstrate clinical efficacy (13). Etanercept (a fusion protein with two TNFα p75 receptors with Fc portion of human IgG1, Enbrel®, Immunex/American Home Products) is also approved for rheumatoid arthritis in the clinic.

IL-1 is also an important component in the pathogenesis of rheumatoid arthritis. It has been shown to induce chemotaxis of neutrophils, lymphocytes and monocytes by increasing expression of both chemokines and adhesion molecules. It enhances the proliferation of fibroblasts leading to pannus formation and stimulates the production of PGE_2 (15, 16). It also contributes to the destruction of bone and cartilage by inducing enzyme production in both synovial fibroblasts and chondrocytes (15, 16). Two types of soluble IL-1 receptors have identified, however, only type I mediates signal transduction. IL-1 type II binds to IL-1

receptors expressed mainly on B-cells, neutrophils and monocytes but does not induce signal transduction. In addition, IL-1 activation in vivo may be modulated by an IL-1 receptor antagonist protein, IL-1ra. It is probably produced and secreted predominantly by neutrophils and monocytes/macrophages in the synovium (16). Initial studies demonstrated a protective effect with IL-1ra in collagen-induced arthritis in mice, however IL-1ra had no effect on a more severe arthritic model, antigen-induced arthritis in mice (17). A possible disadvantage to systemic IL-1ra treatment is the low levels that may be achieved in the inflammed joint. Only ~5% of cell IL-1 receptors need to be occupied by IL-1 in order to achieve cell activation, thus >95% of IL-1 receptors need to be blocked by IL-1ra in order to achieve inhibition. Using gene therapy, delivery of IL-1ra-transfected synoviocytes locally into the inflammed joint resulted in a significant reduction of joint swelling in bacterial cell wall-induced arthritis in rats (18).

Another approach to inhibiting IL-1, is to use soluble IL-1 receptor type II (sIL-1RII) as an intervention. In a rabbit model of antigen-induced arthritis, sIL-1RII treatment significantly reduced joint swelling and bone erosion, though it did not reduce PGE_2 levels in the synovium (19). These experimental findings are also reflected in clinical results obtained to date with the IL-1 receptor antagonist, IL-1ra (anakinra, Amgen). There has been only a small clinical benefit in patients tested so far, however, a signifiant radiological improvement of affected joints could be demonstrated (20).

Combination therapy

These observations in the clinic that the inhibition of IL-1 and TNFα appear to affect aspects of arthritis independent of each other has led to the concept that the combination of each may be additive. In addition, it is theorised that lower doses of each compound can be used than when each one is given alone.

Animal studies looking at these combinations have yielded some interesting results. In a gene therapy model of rabbit antigen-induced arthritis, virus encoding TNFα soluble receptor protein was transfected into the arthritic knee joint and shown to have little effect on leukocyte infiltration into the joint and no effect on cartilage breakdown (21). When virus encoding IL-1 soluble receptor type I-IgG fusion protein was transfected into the affected joint, there was a reduction in both cartilage breakdown and white cell infiltration into the joints. Combination gene therapy with the two soluble receptors resulted in a greater effect than either compound alone. Similarly in another study using murine streptococcal cell wall-induced arthritis (22), anti-TNFα treatment significantly reduced joint swelling, but not IL-1ra. Anti-TNFα treatment also did not affect leukocyte infiltration into the joint or cartilage

damage, however, anti-IL-1, resulted in a significant reduction of leukocyte infiltration into the joint and cartilage damage. Combined treatment suppressed both joint swelling and cartilage damage, but the effects were additive and not synergistic. Actual synergistic effects of IL-1 and TNFα inhibition have been measured in a model of adjuvant arthritis by Feige et al (23). The combination of IL-1ra and pegylated TNF soluble receptor I that had little or no effect alone significantly reduced both inflammation and loss of bone mineral density when given together. In addition, the highest doses of each compound showed a significant inhibition of all parameters measured, including weight loss, joint inflammation and bone mineral density. However, when these doses were administered together, the arthritic disease course was completely reversed.

Thus, pre-clinical experiments suggest that TNFα is more important in the inflammatory portion of the disease, while IL-1 may be more important in later stages of disease, namely bone and cartilage damage. Thus cytokine inhibitors have not only allowed more insight into disease pathology, it has also suggested a more effective course of therapy.

Non Steroidal Anti-Inflammatory Drugs

NSAIDs represent one of the most widely prescribed classes of drugs in the world. Their anti-inflammatory, analgesic and antipyretic properties are so effective that each day thirty million people take some form of NSAID. Previously it was believed the adverse effects that accompany these drugs are inevitable based on our understanding of how NSAIDs work, i.e. the anti-inflammatory effects are inseparable from the gastrointestinal and renal side effects. Vane in 1971 (24) showed that the key enzyme in the synthesis of prostaglandins was cyclooxygenase (COX) and that all NSAIDs act by inhibiting this enzyme. Prostaglandins are important mediators of inflammation, however, also have a variety of physiological functions, which include protection of the gastrointestinal mucosa, maintenance of renal blood flow and regulation of haemostasis. Thus, it was difficult to imagine how certain NSAIDs could be better tolerated than others given comparable therapeutic activity. The discovery that COX exists in two isoforms (25, 26) - COX-1 and COX-2 - brought a new perspective to the relationship between the therapeutic effects and the side effects of NSAIDs.

Inhibitor Selectivity for COX-1 and COX-2

Selective COX-1 inhibitors

Aspirin irreversibly inhibits COX. At low doses used for cardiovascular protection it is a selective COX-1 inhibitor in platelets (27). The absorption of one 100 mg tablet will encounter the entire mass of platelets in the portal circulation, thereby completely inhibiting

COX-1 for the life of the platelet (7-10 days). The concentrations in the systemic circulation are too low to cause meaningful COX inhibition elsewhere. At higher doses COX-1 inhibition can occur in other tissues and, consequently, more gastrointestinal damage results.

Nonselective inhibitors

Most of the conventional NSAIDs inhibit both COX-1 and COX-2 (28). This group includes indomethacin, piroxicam, naproxen, ibuprofen, diclofenac and high dose aspirin. These drugs inhibit platelet aggregation and have anti-inflammatory and analgesic activity in humans but also cause significant gastrointestinal and renal side effects.

Selective COX-2 inhibitors

Selective COX-2 inhibitors include rofecoxib, meloxicam and celecoxib. These drugs inhibit COX-1 between 20 - 60% at therapeutic plasma concentrations in the whole blood assay. In contrast to conventional NSAIDs, these drugs do not inhibit platelet aggregation or bleeding time, the functional COX-1 parameters in humans (29, 30, 31) and more importantly have fewer gastrointestinal adverse events in the clinic.

COX-2 Involvement in Inflammation and Pain

The principal difference between COX-1 and COX-2 lies in their tissue distribution profile and the way they are regulated. COX-1 is a constitutive enzyme present in most tissues, including gastrointestinal mucosa, kidney, platelets, brain, liver and spleen. COX-1 is responsible for the synthesis of prostaglandins with "physiological" functions. The COX-2 isoenzyme is inducible. COX-2 is not expressed under basal conditions, except in some organs (brain, spinal cord and kidney) where it is expressed constitutively. However, in response to inflammatory stimuli such as lipopolysaccharide or pro-inflammatory cytokines (IL-1, TNF-α) its expression is induced in a variety of cells such as monocyte/macrophages, endothelial cells and synoviocytes (32). This induced expression is tightly regulated by cytokine expression. In IFN-γ primed macrophages, full expression of COX-2 mRNA occurs in response to LPS or TNFα stimulation (33). IL-1 inhibition by specific antibodies has also been shown to reduce COX-2 expression and PGE_2 production (32). In addition, TNFα, IL-1 and IL-8 can induce COX-2 expression in neutrophils (34). PGE_2 can also induce IL-6 expression and may be involved its regulation (35). COX-2 expression is also inhibited by anti-inflammatory cytokines such as IL-4, IL-10 and IL-13, TGFβ and by glucocorticoids (36, 37).

In vivo, COX-2 has been shown to be present at elevated concentrations at sites of inflammation both in animal models and in the synovia of patients with arthritis (38). In a model of carrageenan-induced inflammation, it was demonstrated that COX-2 mRNA and

protein was upregulated and PGE_2 levels were elevated in the inflammed paw (39). In addition, increased expression paralleled the time course of inflammation, and was reduced by the administration of indomethacin. COX-2 immunoreactivity was localised to the inflammatory cells. The role of PGE_2 in this process has also been highlighted by Portanova et al (40), who inhibited carrageenan-induced edema in rats with a monoclonal antibody against PGE_2. Moreover, the selective COX-1 inhibitor, SC-560, has been shown to be inactive in a rat model of carrageenan-induced inflammation (41). These data underscore the role of COX-2 in inflammatory processes.

Pharmacological data obtained in various animal models of inflammation demonstrate that the anti-inflammatory activity of selective COX-2 inhibitors is comparable to that of non-selective NSAIDs. Adjuvant arthritis in rats is induced by the injection of heat killed mycobacteria in suspension with Freunds adjuvant into the right hind foot pad (42). Treatment is usually begun simultaneously with induction of arthritis, and is given daily for the duration of the experiment, which routinely is 3 weeks. The most frequent outcome measures are degree of inflammation at the primary site of injection and of the contralateral paw. Radiological scores of joint integrity are performed and general markers such as weight loss, spleen weight and erythrocyte sedimentation rates are performed.

Meloxicam, celecoxib and rofecoxib have all been tested in this model of chronic inflammation (43, 44, 45). All were given in increasing doses once daily, except for celecoxib, which was given twice daily. Dosing of celecoxib was also started on day 15 after onset of injury, the others were administered on day 0. Rofecoxib resulted in an ID_{50} of 0.7 mg/kg/day after 21 days of treatment, celecoxib resulted in an ID_{50} of 0.37 mg/kg/day and meloxicam 0.12 mg/kg/day. These are compared to indomethacin in this model, which routinely has an ID_{50} of ~0.8 – 1.0 mg/kg/day. Thus, these COX-2 selective inhibitors are as effective or more effective than indomethacin in this model. Similar results were obtained with the carageenan-induced paw edema model.

COX-2 inhibitors also appear to be as effective as nonselective NSAIDs in arthritic diseases in the clinic (46-49). Approved indications for oral formulations include osteoarthritis (meloxicam, celecoxib, rofecoxib), rheumatoid arthritis (meloxicam, celecoxib) and ankylosing spondylitis (meloxicam).

Analgesic activity of selective COX-2 inhibitors

NSAIDs are generally classified as being effective against mild-to-moderate pain, but this classification is not completely accurate. For the assessment of analgesic efficacy, the type of pain should also be considered. NSAIDs are particularly effective in conditions in which

inflammation or tissue injury has caused sensitization of pain receptors. Under these conditions COX-2 is elevated and NSAIDs most probably exert their analgesic effects via this mechanism.

In carrageenan-induced hyperalgesia in the rat, increased expression of COX-2 was seen both at the site of inflammation and in the spinal cord, however, COX-1 was also expressed at both of these sites. Standard NSAIDs and selective COX-2 inhibitors (43-45), but not a selective COX-1 inhibitor (41), were found to inhibit or reverse hyperalgesia in this model. Thus, COX-2 appears to be the principal isoenzyme involved in models of inflammatory pain, however, there is evidence that COX-1 appears to be the predominant isoenzyme involved in acute models of chemically-induced irritation.

Selective COX-2 inhibitors are effective pain relievers in different clinical settings. Symptomatic relief of pain has been approved in osteoarthritis (meloxicam, celecoxib, rofecoxib), rheumatoid arthritis (meloxicam, celecoxib) and ankylosing spondylitis (meloxicam). In addition, treatment of acute pain and dysmenorrhea is approved for rofecoxib and celecoxib and the management of back-pain and sciatica as well as pain conditions resulting from scapulohumeral periarthritis and neck-shoulder-arm-syndrome for meloxicam.

COX inhibition and gastrointestinal toxicity

Cytoprotective prostaglandins are produced by COX-1 in the gastric mucosa (50). This could explain the higher gastrointestinal adverse events seen with nonselective inhibitors as compared to COX-2 selective inhibitors. Large-scale clinical trials of meloxicam (46, 47), celecoxib (48) and rofecoxib (49) all show improved gastrointestinal tolerability and a low incidence of gastric perforations, ulcers and bleeding.

Additionally, the most common gastrointestinal complication is bleeding. COX-2 inhibitors are COX-1 sparing and greatly reduce gastrointestinal bleeding because they preserve platelet function. Recently it has been demonstrated that co-use of aspirin may reduce this advantage of COX-2 selective inhibitors (48). Appropriate management of high-risk gastrointestinal patients remains uncertain with both these inhibitors and nonselective NSAIDs since this has not been studied appropriately in these patient populations (i.e. those with an active ulcer or a recent history of ulceration). Caution is particularly warranted because data from experimental studies suggest that COX-2 may be important in the healing of gastric ulcers. In addition, pharmacovigilance studies will become important for determining the safety of selective COX-2 inhibitors in long term therapy.

Conclusions

Recognition of the shortcomings of current antirheumatic therapy has triggered the development of new therapies for the treatment of rheumatoid arthritis. At the same time, an explosion of information concerning the biology and pathogenesis of rheumatoid arthritis, obtained in vitro and in animal models, has led to the identification of numerous potential targets for therapeutic intervention. It is becoming clear from this information that rheumatoid arthritis is a multidimensional disease. Not only may treatment efficacy be different in different patients, it is also becoming clear that administering combinations of safer drugs that target the various stages of rheumatoid arthritis is probably the method of choice in the future. In this way, different treatments can be combined and tailored to each patient depending on individual disease status.

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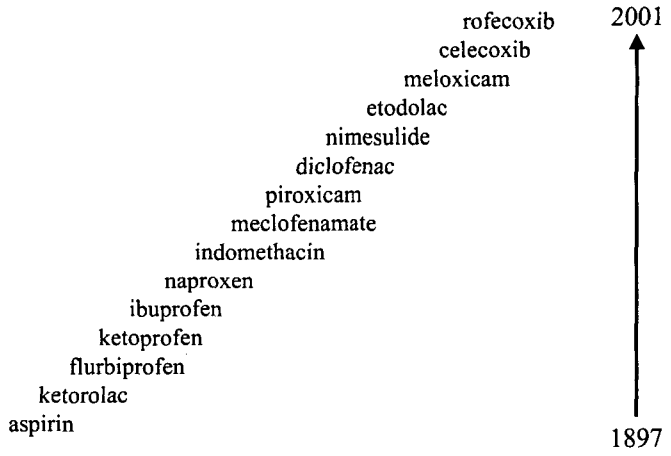
10. Wallace JL, McKnight W, Reuter BK *et al.* NSAID-induced gastric damage in rats: Requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000; 119:706-714.
15. Swan SK, Rudy DW, Lasseter KC et al. Effect of cyclo-oxygenase-2 inhibition on renal function in elderly persons receiving a low-salt diet. Ann Intern Med 2000; 133:1-9.

Animal Models of Arthritis: Pharmacological Interventions

Korean Society of Applied Pharmacology
Seoul, Korea

JvR 11-01 1

Non Steroidal Anti-Inflammatory Drugs



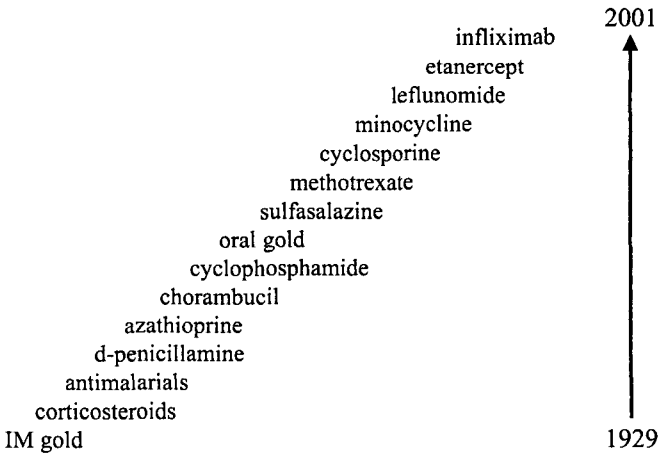
JvR 11-01 2

Rheumatoid Arthritis

- Common, chronic, incurable disease
  - affects 1% of population world-wide
  - characterized by chronic inflammation and joint destruction
  - initial cause of disease unknown
  - 80% patients disabled after 20 years
  - life expectancy reduced 3-18 years
  - costs ~ $6000 per patient per year

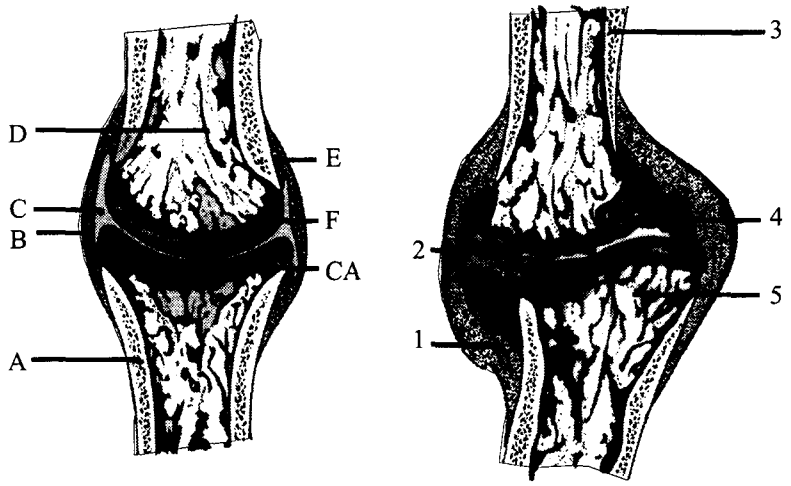
JvR 11-013

Disease Modifying Anti-Rheumatic Drugs



JvR 11-014

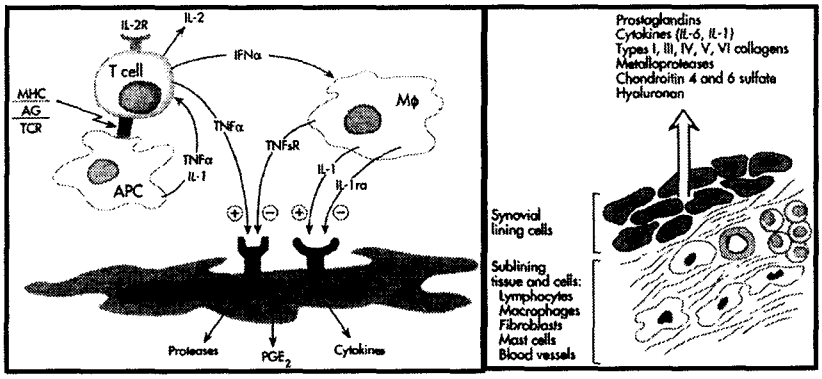
Normal and Rheumatoid Joint



From: ED Harris, Rheumatoid Arthritis, WB Saunders, 1997.

JvR 11-01 5

Pathophysiology of Rheumatoid Arthritis

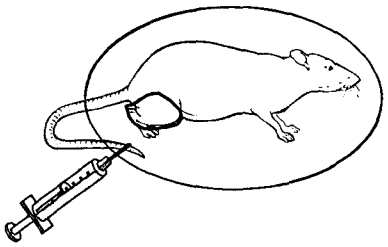


From: ED Harris, Rheumatoid Arthritis, WB Saunders, 1997.

JvR 11-01 6

Mouse Collagen-Induced Arthritis

Day 0: Induction of arthritis, collagen type II and Freunds adjuvant, intradermal



Prevention:
Treatment begins Day 0
Clinical Severity
- Incidence of arthritis
- Day of onset
- Clinical score

Maximum paw swelling
- Histopathology
- Total number of joints
- Degree of swelling

Anti-collagen IgG

Treatment:
Treatment begins after onset
Arthritis onset 2-4 weeks
- Clinical score

Paw swelling
- Histopathology
- Degree of swelling

Anti-collagen IgG

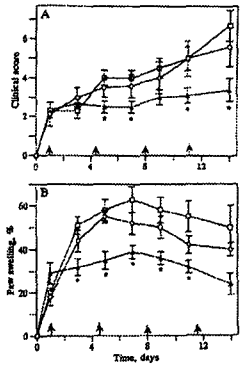
JvR 11-017

Anti-TNF Antibody (250 μg per mouse)

Prevention
(one treatment per week for 4 weeks)

	TN3-19.12	PBS
Clinical Severity:		
Incidence	6/9	8/10
Day of onset	29.0	29.6
Clinical score	3.7	4.4
Paw swelling (%)	30	51
Histopathology:		
Joints assessed	16	20
Severe	3 (19%)	13 (65%)

Treatment
(twice per week for 2 weeks after onset)



Williams, Feldmann, Maini, PNAS 89:9784-9788, 1992.

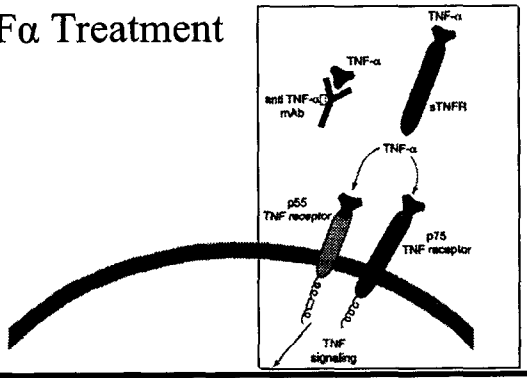
JvR 11-018

Anti-TNF Treatment in Arthritis

- The anti-inflammatory importance of TNFα has been confirmed by others in animal models (Wooley et al J Immunol 151:6602-6607, 1993)
- Transgenic mice expressing TNFα develop chronic inflammatory polyarthritis and this is inhibited by anti-TNF antibodies and IL-1 receptor antagonists (Probert et al, Eur J Immunol 25:1794-1797, 1995)
- Clinical success with TNFα inhibitors in rheumatoid arthritis

JvR 11-01 9

Anti-TNFα Treatment



Signal cascade

Stimulates production and release of metalloproteinases (i.e. collagenases stromelysin, PGE_2, nitric oxide)

Promotes bone absorption

Increases production of proinflammatory cytokines

Inhbits production of matrix components

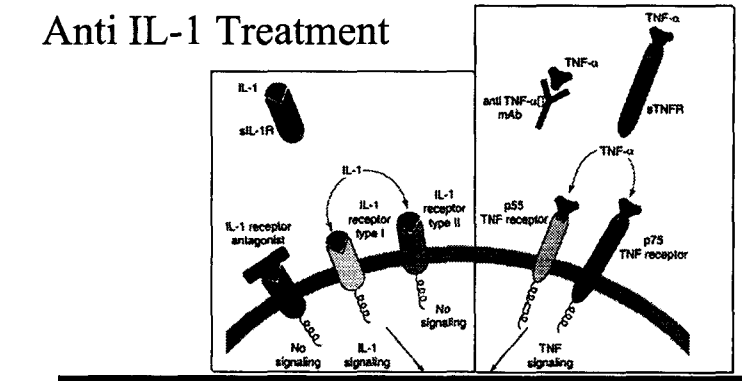
Degradation of cartilage and tissue matrix and recruitment of in flammatory cells

Inflammation and Joint destruction

Carteron, Mol Med Today 6:315-323, 2000

JvR 11-01 10

Anti IL-1 Treatment



Signal cascade

Stimulates production and release of metalloproteinases (i.e. collagenases stromelysin, PGE_2, nitric oxide)

Promotes bone absorption

Increases production of proinflammatory cytokines

Inhbits production of matrix components

Degradation of cartilage and tissue matrix and recruitment of in flammatory cells

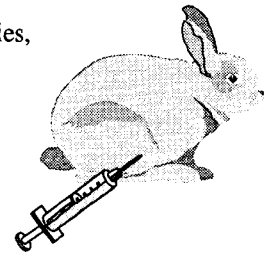
Inflammation and Joint destruction

Carteron, Mol Med Today 6:315-323, 2000

JvR 11-01 11

Rabbit Antigen-induced Arthritis

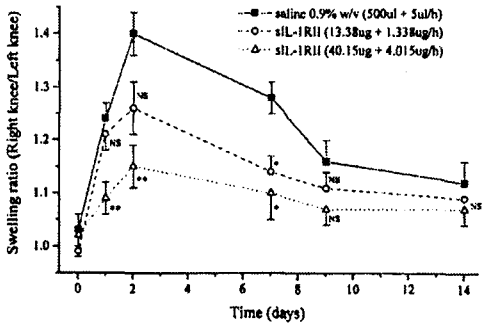
Day -28 & -14:	Rabbits are sensitized to mBSA in Freunds Adjuvant via intradermal injection
Day 0:	Rabbits anesthetized, right knee mBSA, left knee 5% glucose
Day 1, 2, 7, 9, 14:	Measure knee joint diameter on both sides
Day 14:	Animals killed, Synovial fluid samples, Histology



JvR 11-01 12

Soluble IL-1 type II receptor

- Mini pumps were surgically implanted on day 0
- Day 0 bolus plus continual sc infusion over 2 weeks
- sIL-1RII showed a dose-dependent inhibition of swelling and a non significant trend to reduced cell infiltrates
- no reduction of joint PGE_2



Dawson et al. Rheumatology 1999; 38:401-406

JvR 11-01 13

Combination Therapy

- "No single drug can inhibit all stages of rheumatoid disease"
- IL-1ra (Anakinra, Amgen) reduced clinical symptoms ~10-20% but showed significant improvement in radiological findings
- TNFα is very effective in reducing inflammation and pain but effects on long term disease progression unknown

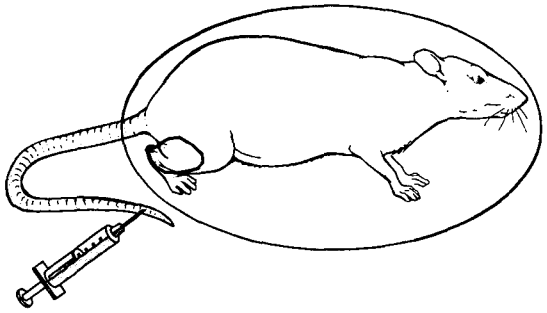
JvR 11-01 14

Rat Adjuvant Arthritis

Day 1: Induction of arthritis, heat-killed Mycobacteria emulsion in oil or Freund's adjuvant, intradermal injection in tail

Day 9 : Onset of arthritis and first day of treatment

Parameters: body weight
mobility
paw volume
radiologic assessment
histology: joint and main organs



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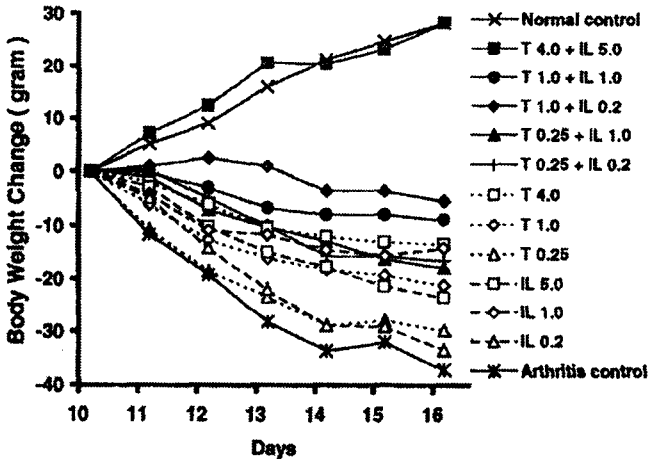
Effects of Combination Therapy

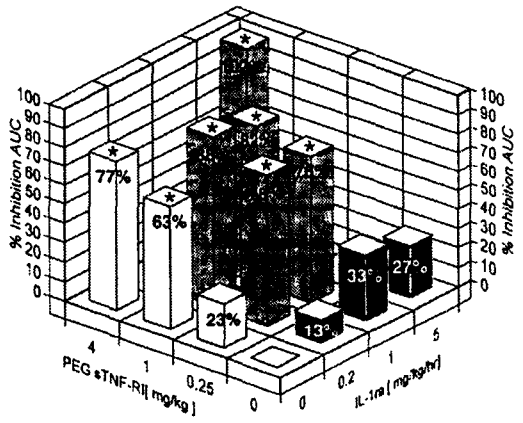


Feige et al. Cell Mol Life Sci 57: 1457-1470, 2000

JvR 11-01 16

Effects of Combination Therapy

Percent Inhibition of Paw Swelling (plethysmography) as AUC over 1 week

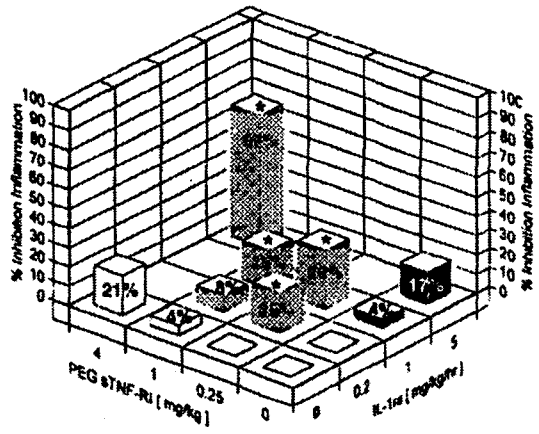


Feige et al. Cell Mol Life Sci 57: 1457-1470, 2000

JvR 11-01 17

Effects of Combination Therapy

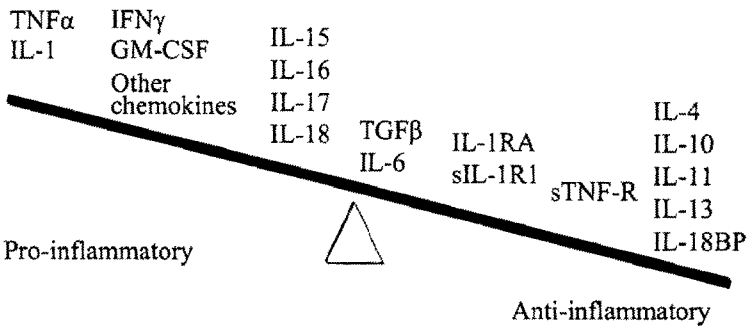
Histopathological score measured as mean reduction compared to adjuvant control



JvR 11-01 18

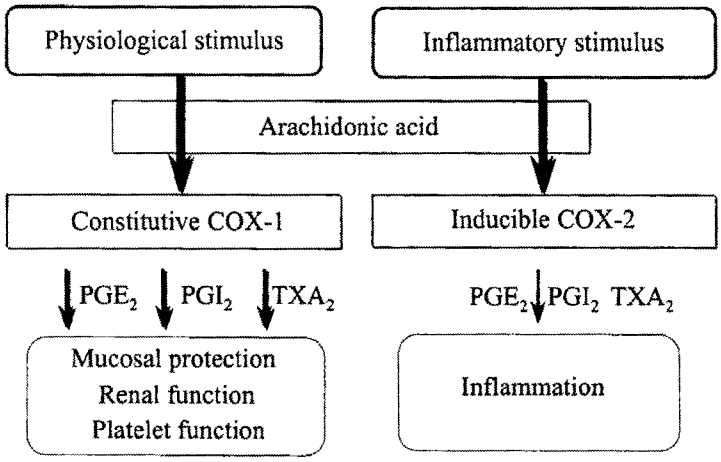
Imbalance in Inflammatory Processes

- Goal of therapy is to restore the inflammatory equilibrium



JvR 11-01 19

COX-1 and -2: Conversion of AA to Prostaglandins



Vane. Nature 1994; 367: 215-216

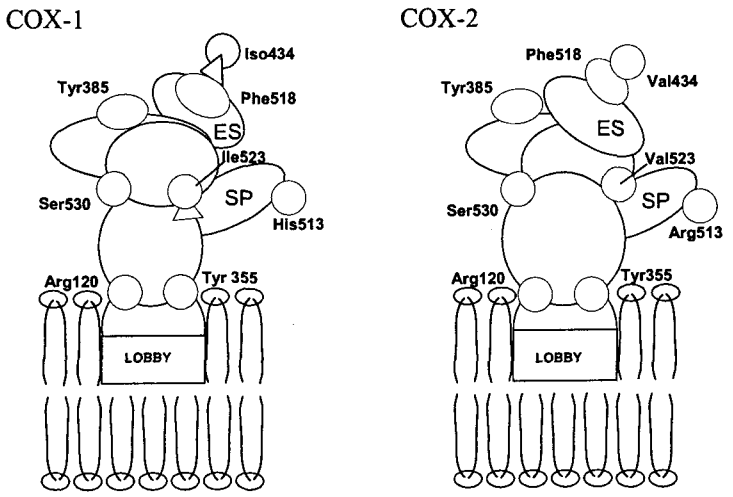
JvR 11-01 20

Structure of COX-1 and COX-2

	COX-1	COX-2
cDNAs	chromosome-9	chromosome-1
	22 kB	8.3 kB
~~mRNAs~~	~~2.8 kB~~	~~4.5 kB~~
Proteins (after cleavage ~~of signal peptides~~)	576 amino acids glycosylated ~~1 mol heme~~	587 amino acids glycosylated ~~1 mol heme~~
Regulation	constitutive	inducible
Homology	amino acids: 85-90% between species for both enzymes COX-1 and COX-2 ~ 60 - 65% identical, similar V_{max} and K_m values for arachidonic acid	
Active site (COX channel) ~~residue differences~~	Ile523 (first shell) ~~His513,Phe503, Ile434~~	Val523 (first shell) ~~Arg513, Leu503,Val434~~

JvR 11-01 21

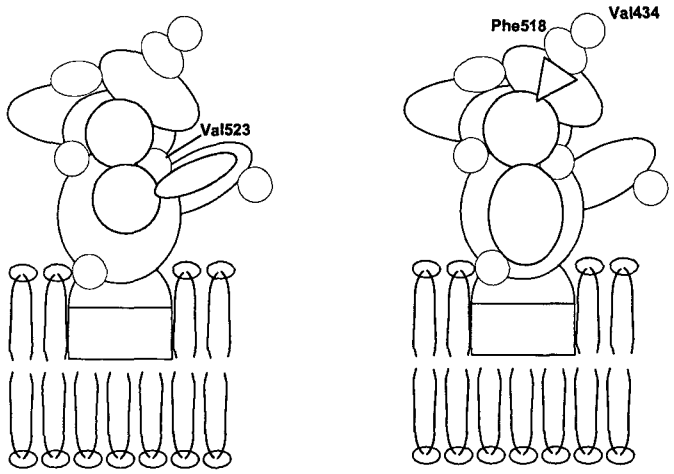
Amino acid substitutions between COX-1 and COX-2. The hatched area in COX-1 are areas more accessible in COX-2 due to the amino acid substitutions



SP: Side Pocket
~~ES: Extra Space~~

JvR 11-01 22

Inhibition of COX-2 by celecoxib (cyanogen) and meloxicam (gold)



JvR 11-01 23

Pharmacology of Selective COX-2 Inhibitors

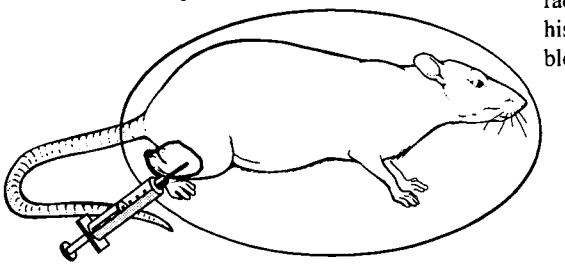
- *in vivo* Pharmacology:
  Efficacy and Safety in Animals
- *ex vivo* Pharmacology:
  Inhibition of PG-Synthesis at Tissue Level in Animals
- *in vitro* Biochemistry:
  COX-2 Selectivity at Enzyme Level in Man

JvR 11-01 24

Rat Adjuvant Arthritis

Day 1: Induction of arthritis, 1 ml heat-killed Mycobacteria in Freund's adjuvant, subplantar injection

Day 1 to 21: Administer test compound



Parameters: body weight
paw volume
relative weight of the liver/spleen
plasma protein electrophoresis
blood sedimentation
plasma IL-6
radiologic assessment
histology: joint and main organs
blood urea, creatinine

JvR 11-01 25

Effect on the Adjuvant-induced Primary and Secondary Inflammatory Reactions in Rats

NSAIDs	ID_{50} (mg/kg/day) Primary reaction	Secondary reaction
Meloxicam	**0.17** (0.14-0.21)	**0.12** (0.09-0.14)
Piroxicam	**0.61** (0.49-0.78)	**0.67** (0.50-0.95)
Diclofenac	**0.97** (0.75-1.36)	**1.24** (0.84-2.68)
Naproxen	**14.3** (10.1 - 18.8)	**11.8** (8.12-14.9)

Engelhardt: Inflamm Res 44, 423-433 (1995)

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Effect of Selective COX-2 inhibitors on Adjuvant Arthritis in rats: Secondary Reaction

Meloxicam[1]:	Rofecoxib[2]:	Celecoxib[3]:
dosing: once daily treatment: 21 days	dosing: once daily treatment: 21 days	dosing: twice daily treatment: day 14 - 24
ED_{50}: 0.12 mg/kg/day	ED_{50}: 0.74 mg/kg/day	**ED_{50}:** **0.37 mg/kg/day**
indomethacin: 0.67 mg/kg/day piroxicam: 0.77 mg/kg/day	indomethacin: 0.7 mg/kg/day	indomethacin: 0.11 mg/kg/day piroxicam: 0.15 mg/kg/day

[1]*Engelhardt. Inflamm Res 44, 423-433 (1995)*
[2]*Chan et al. J Pharmacol Exp Ther 290 (2) 551-560 (1999)*
[3]*Penning et al. J Med Chem 40, 1347-1365 (1997)*

JvR 11-01 27

Analgesic (antinociceptive) and antipyretic actions

Meloxicam:	Rofecoxib:	Celecoxib:
analgesic action AA, rats, 5th day[1]:	carrageenan-induced hyperalgesia, rats s.d.[2]:	effective in various models of analgesic and antipyretic activity[4]
ED_{50}: 0.4 mg/kg	ED_{50}: 1.0 mg/kg	
LPS-induced pyresis, cats, s.d., 5 hrs[2]:	LPS-induced pyresis, rats, s.d., 5 hrs[3] :	
ED_{50}: 0.1 - 0.3 mg/kg	ED_{50}: 0.24 (+0.07) mg/kg	

Additional involvement of COX-1 in pain and role of COX-2 in CNS

[1]*Laird et al. Inflamm Res 46, 203-210 (1997)*
[2]*Justus et al. Vet Res Commun 19, 321-330 (1995)*
[3]*Chan et al. J Pharmacol Exp Ther 290 (2) 551-560 (1999)*
[4]*Penning et al. J Med Chem 40, 1347-1365 (1997)*

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Anti-Inflammatory Potency (AA) and Ulcerogenic Potency in Rats

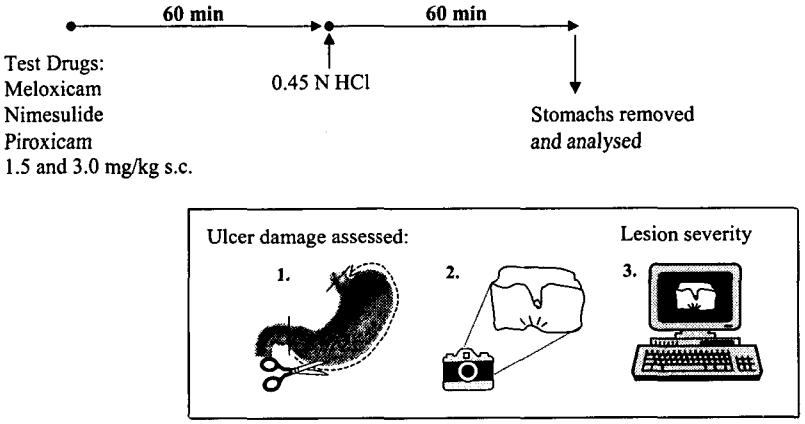
	Adjuvant Arthritis (ID_{50})	Stomach Ulcer (ED_{50})	Ratio Ulcer/Arthritis
Meloxicam	0.12 (0.09-0.14)	2.42 (1.64-3.56)	20
Diclofenac	1.24 (0.84-2.76)	2.71 (2.38-3.09)	2.2
Piroxicam	0.77 (0.46-1.71)	1.09 (0.26-1.41)	1.4
Naproxen	11.8 (8.1-14.9)	11.2 (8.1-15.4)	1.0

(95% confidence limits)

Engelhardt: Br J Rheumatol 35 (Suppl 1), 4-12 (1996)

JvR 11-01 29

Acid-Induced Gastric Lesions



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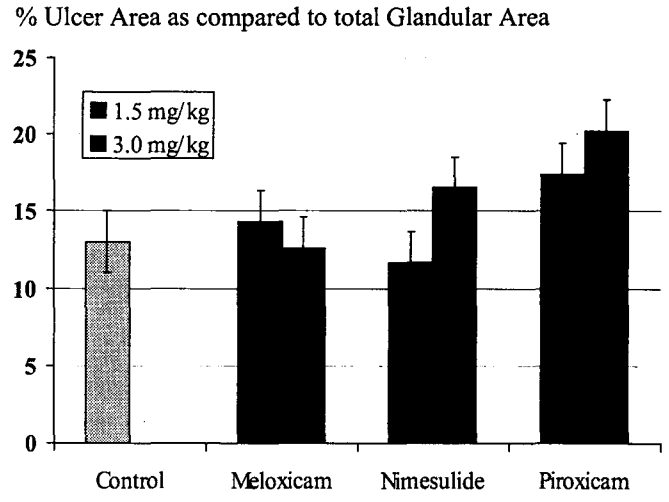
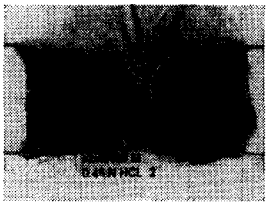
Effect on Developing Gastric Lesions in Rats



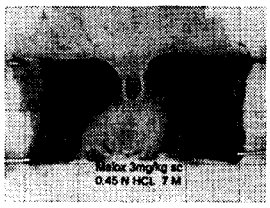
van Ryn, Schierok, J. Rheumatol. 28 (suppl 63):5;2001

JvR 11-01 31

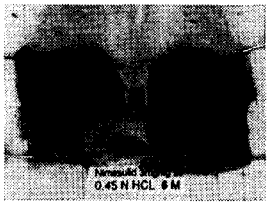
Effects on Developing Gastric Lesions in Rats



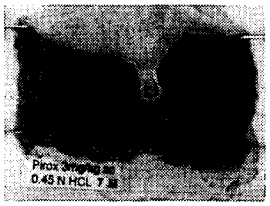
Placebo:
13% ulcer area



Meloxicam 3 mg/kg:
13% ulcer area



Nimesulide 3 mg/kg:
16% ulcer area



Piroxicam 3 mg/kg:
22% ulcer area (<0.05 vs. mel)

van Ryn, Schierok, J. Rheumatol. 28 (suppl 63):5;2001

JvR 11-01 32

COX-2 Inhibitors: Ulcerogenic Potential in Rats

	Meloxicam	Rofecoxib
Sub-acute GI-ulcers	UD_{50}: 2.42 mg/kg[1]	no lesions at 300 mg/kg[3]
GI-permeability, Cr^{51}rbc	n.d.	no effect at 300 mg/kg[3]
Chronic GI-ulcers 52 w	< 1 mg/kg[2]	< 2 mg/kg (f)[4]
	NOAEL: 0.8 mg/kg[2]	NOAEL: 1.0 mg/kg[4]
Comparison ED_{50} AA	0.12 mg/kg	0.74 mg/kg

Celecoxib: NOAEL not achieved in 2 y study[5]

[1] *Engelhardt. Inflamm Res 44, 423-433 (1995)*
[2] *Lehmann. Inflammopharmacology 4, 105-123 (1996)*
[3] *Chan et al. J Pharmacol Exp Ther 290 (2) 551-560 (1999)*
[4] *Rofecoxib Summary Basis of Approval 021042/52, FDA 5/20/99, p. 88-97*
[5] *Celecoxib Summary Basis of Approval 20998, FDA 12/31/98, p.43-51*

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Summary: *In vivo* pharmacology
Efficacy and safety in animals

- COX-2 inhibitors are potent anti-inflammatory, analgesic and antipyretic agents
- Gastric side effects of COX-2 inhibitors is reduced as compared to non-selective NSAIDs
- Ulcerogenicity of COX-2 inhibitors may be a function of dose and duration of exposure

JvR 11-01 34

Inhibition of PG-Synthesis at the Tissue Level in Animals

⇨ Inhibition of PG-synthesis after oral administration in inflammatory tissues (induced COX-2) and in tissues with physiological PGs (constitutive COX-1)

caveats:

* Pharmacokinetics influence results
* Non-selective inhibitors show variable ID_{50} in different tissues
* What is the degree of PG inhibition required for functional effects: ID_{50} vs. ID_{80}? ID_{95}?

JvR 11-01 35

Rat Air Pouch Model

Pouch Formation:

Day 1: 20 ml of sterile air sc

Day 3: additional 10 ml air

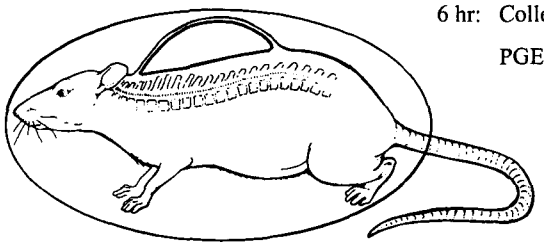
Induction of inflammation

Day 6: -1 hr: Administer test compound

0 hr: Inject carrageenin into pouch

6 hr: Collection of pouch exudates

PGE_2 content measured



JvR 11-01 36

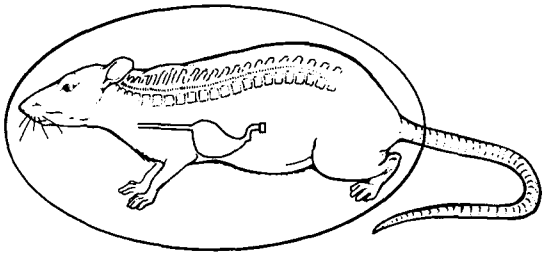
Rat Gastric PGE_2

0 min: Administer test compound

90 min: Pylorus ligation

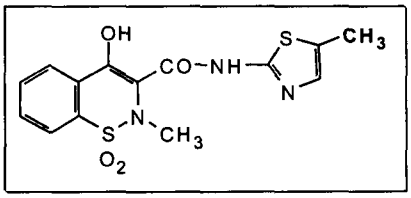
4 hr + 90 min: Collection of gastric juice

PGE_2 content measured

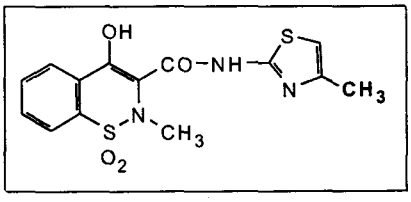


JvR 11-01 37

Selection of a COX-Inhibitor Analogue



meloxicam (COX-2 inhibitor)



4'-meloxicam
(dual COX-1/COX-2 inhibitor)

AA	ED_{90}	0.1 mg/kg	0.2 mg/kg
Ulcer	ED_{50}	2.4 mg/kg	0.4 mg/kg

4'-meloxicam: very minor structural change results in a different pharmacological class

Pairet et al. Inflamm Res 47, 270-276 (1998)

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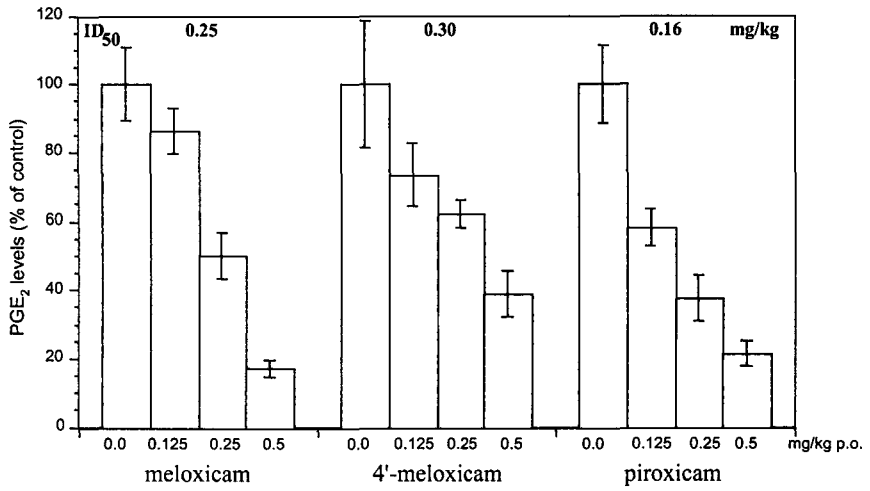
Dose Response Measurements for PGE_2 Synthesis in Air Pouch Exudate in Rats



Pairet et al. Inflamm Res 47, 270-276 (1998)

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Tissue selective inhibition of PGE_2 by meloxicam

	inflammatory PGE_2 ("air pouch") ED_{50} (mg/kg)	gastric PGE_2 ("shay rat") ED_{50} (mg/kg)	renal PGE_2 ("water-loading") ED_{50} (mg/kg)
meloxicam	0.25	27	1.92
4'-meloxicam	0.30	4.1	0.46
piroxicam	0.16	1.7	0.47

Pairet et al. Inflamm Res 47, 270-276 (1998)

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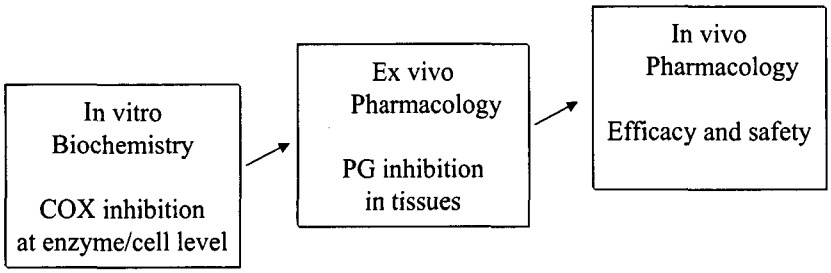
Summary: *Ex vivo* pharmacology
Inhibition of PG-synthesis at tissue level in animals

- Favourable GI-safety of meloxicam in rats can be explained by selective PG-inhibition
- *Ex vivo* models generally can be used to bridge *in vivo* data with *in vitro* selectivity measurements



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COX-2 Selectivity using Recombinant Human COX

- Initial estimates for COX-2 selectivity were very high
- IC_{50} depend on experimental conditions
- IC_{50} values generally do not reflect drug concentrations in man

[1] *Churchill et al. Inflammopharmacology 4, 125-135 (1996)*
[2] *Kremer. J Rheumatol 27 (Suppl 60) 9-12 (2000) / Penning et al. J Med Chem 40, 1347-1365 (1997)*
[3] *Chan et al. J Pharmacol Exp Ther 290 (2) 551-560 (1999)*

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Tests for COX-2 selectivity

- **Purified enzyme or cell lines with expressed COX:**
  - suitable for screening
- **For clinical relevance:**
  - whole blood assays with human blood
  - compare *in vitro* IC_{50} values to drug concentration *ex vivo*
  - determine *ex vivo* COX inhibition in man

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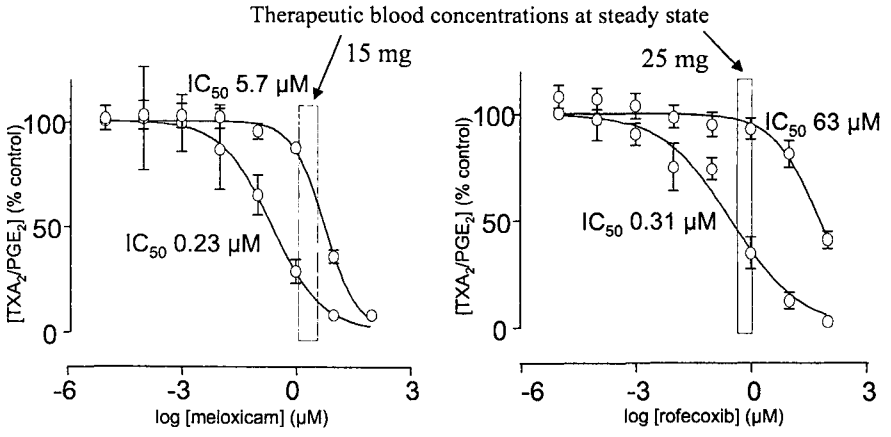
Inhibition by Meloxicam and Rofecoxib of COX-1 & COX-2 in human blood/A549 cells



Warner et al. Proc Natl Acad Sci USA 96, 7563-7568 (1999)

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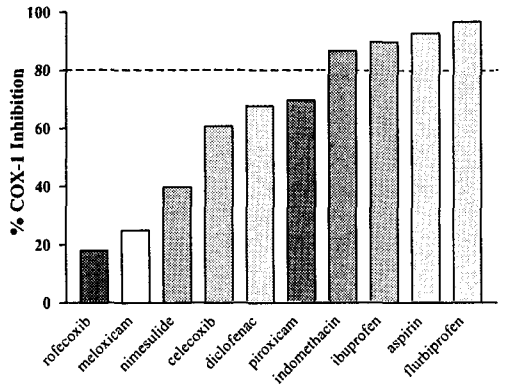
COX-1 Inhibition when COX-2 Inhibition is 80% (i.e. therapeutic)



Warner et al. PNAS 96:7563-7568 (1999)

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Summary

- Selective COX-2 inhibitors present a pharmacological profile distinct from the conventional NSAIDs
- COX-2 inhibitors consistently shows favourable GI-safety in pre-clinical pharmacology
- Therapeutic doses of COX-2 inhibitors do not affect platelet aggregation and bleeding time in man

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