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INFLAMMOPHARMACOLOGY 2003

*Royal College of Physicians of Edinburgh
Edinburgh, 22–24 April 2003*

Programme and Abstracts

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- Professor George Nuki (Western General Hospital, University of Edinburgh, Scotland, UK)

PROGRAMME***Theme: Pain in Osteoarthritis***

Numbers in parentheses denote the number of the Abstract

Tuesday 22 April
Morning Session
0800 Registration

0830–0835 Welcome by Chairmen/Organisers

SESSION 1: Osteoarthritis — Aetiology and Clinical Manifestations

Chair: Prof. Kim Rainsford and Dr. Michael Powanda

0835–0900

(1) Osteoarthritis: symptoms and signs

Prof. Walter Kean, McMaster University, Hamilton, Canada

0900–0930

(2) Cartilage cell biology of osteoarthritis

Prof. Linda Sandell, Washington University School of Medicine, St. Louis, MO, USA

0930–0955

(3) Regulation of bone lysis in inflammatory diseases

Dr. David Haynes, University of Adelaide, Adelaide, SA, Australia

0955–1020

(4) Vascular mechanisms in osteoarthritis and relevance to treatment agents

Dr. Philip Cheras, University of Queensland, Brisbane, Qld, Australia

1020–1045

(5) Molecular regulation of chronic inflammation

Prof. Jack Gauldie, McMaster University, Hamilton, Canada

1045–1110 Coffee/Tea

1110–1135

(6) Experimental pain models — mechanisms of pain

Dr. Steve Gauldie, University of Edinburgh, Edinburgh, UK

1135–1200

(7) Health status assessment in research and clinical practice environments in osteoarthritis

Prof. Nick Bellamy, University of Queensland, Brisbane, Qld, Australia

1200–1225

(8) The impatient patient: a personal view of osteoarthritis

Dr. Michael Powanda, M/P Biomedical Consultants LLC, Mill Valley, CA, USA

1225–1330 Lunch

Afternoon Session

SESSION 2: OSTEOARTHRITIS — Therapeutics

Chair: Prof. Laurie Prescott and Prof. Walter Kean

1330–1355

(9) Therapy of pain in osteoarthritis — no drugs?

Prof. Paul Dieppe, Medical Research Council and University of Bristol, Bristol, UK

1355–1420

(10) Global treatments for osteoarthritis

Prof. George Ehrlich, University of Pennsylvania, Philadelphia, PA, USA

1420–1445

(11) NSAIDs and opioids in the treatment of osteoarthritis

Dr. Géza Bálint, National Institute of Rheumatology and Physiotherapy, Budapest, Hungary

1445–1510

(12) Meloxicam, a COX-2 selective NSAID: what's a nice drug like you doing in a class like this?

Dr. Albert Agro, Boehringer-Ingelheim Canada, Burlington, Ontario, Canada

1510–1535

(13) Dexibuprofen: pharmacology, therapeutic uses and safety

Dr. Stefan Kaehler, Gebro Pharma GmbH, Fieberbrunn, Austria

1535–1600 Tea/Coffee

1600–1625

(14) Place of OTC analgesics and NSAIDs in self-medication of osteoarthritis

Prof. Nicholas Moore, University of Bordeaux II, Bordeaux, France

1625–1650

(15) Assessment of the safety of COX-2-selective drugs

Prof. Richard Hunt, McMaster University, Hamilton, Ontario, Canada

1650–1730

DEBATE: COX-2 selective drugs, NSAIDs versus analgesics in osteoarthritis; is some COX-1 inhibition desirable for analgesia?

Chair: Prof. Kim. Rainsford

All participants

1800–1930 Reception

Wednesday 23 April
Morning Session
0800 Registration

SESSION 3: Osteoarthritis — Emerging Developments

Chair: Prof. George Nuki and Prof. George Ehrlich

0820–0845

(16) Current strategies and emerging developments

Prof. Frank Wollheim, Lund University Hospital, Lund, Sweden

0845–0910

(17) Phase-III results of licofelone (ML-3000), an inhibitor of COX-1, COX-2 and 5-LOX, in osteoarthritis and endoscopic studies

Prof. Stefan Laufer, University of Tübingen, Tübingen, Germany

0910–0935

(18) Percutaneous analgesics and NSAIDs in osteoarthritis

Prof. Michael Roberts, University of Queensland, Brisbane, Qld, Australia

0935–1000

(19) Pharmacology and therapeutics of drugs used to treat osteoarthritis in veterinary practice

Prof. Peter Lees, Royal Veterinary College, London, UK

Cardioprotection from NSAIDs

1000–1025

(20) Inhibition of the clinical benefits of aspirin on first MI by non-steroidal anti-inflammatory drugs

Prof. Michael Gaziano, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

1025–1100 Coffee/Tea

1100–1125

(21) The cardiovascular effects of concurrent non-selective anti-inflammatory drugs and aspirin

Dr. Marie Hudson, McGill University Health Centre, Montreal, Quebec, Canada

1125–1150

DISCUSSION: Place of NSAID treatments in patients at risk of cardiovascular disease. All participants

SESSION 4: Osteoarthritis — Novel Agents and Procedures

Chair: Prof. George Nuki and Prof. George Ehrlich

1150–1215

(22) Inhibitors of iNOS: potential for symptomatic relief and disease modification in osteoarthritis

Dr. Nigel Boughton-Smith, AstraZeneca, Charnwood, UK

1215–1315 Lunch

Afternoon Session

NO-donating NSAIDs

Chair: Dr. Gordon Letts and Dr. Nigel Boughton-Smith

1315–1350

(23) Nitric oxide, inflammation and NSAID-induced gut injury

Prof. Brendan Whittle, William Harvey Research Institute, St Bartholomew's and Royal London School of Medicine, London, UK

1350–1415

(24) New COX-2-selective CINODs

Dr. Gordon Letts, Nitromed Inc, Boston, MA, USA

1415–1440

(25) A new paradigm in pain and inflammation treatment: COX-inhibiting nitric oxide donator (CINOD)

Dr. Janet Hoogstraate, AstraZeneca R&D, Södertälje, Sweden

1440–1505

(26) The CINOD, AZD3582, exhibits improved gastrointestinal safety compared with naproxen in healthy volunteers

Dr. Bror Jonzon, AstraZeneca R&D, Södertälje, Sweden

1505–1520 Tea/Coffee

SESSION 5: Osteoarthritis: Newly-emerging Therapies

Chair: Dr. Gordon Letts and Prof. Bill Dawson

1520–1550

(27) Mechanical signal-transduction pathways in cartilage and chondrocytes in osteoarthritis — New targets for therapy?

Prof. George Nuki, Western General Hospital, University of Edinburgh, Edinburgh, UK

1550–1615

(28) Heat-shock proteins and their role in chondrocyte protection, an application for autologous transplantation

Dr. Michael Seed, William Harvey Research Institute, London, UK

1615–1640

(29) Combination therapy for inflammation: synergism of NSAIDs/steroids with some herbal/animal products

Dr. Michael Whitehouse, University of Queensland, Brisbane, Australia

1640–1705

(30) The effects of opioids and cannabinoids on chondrocytes

Dr. Rowena Bunning, Sheffield Hallam University, Sheffield, UK

(31) Effects of NSAIDs on cartilage in osteoarthritis

Prof. Kim Rainsford, Sheffield Hallam University, Sheffield, UK

SESSION 6: Novel Aspects of Chronic Diseases

Chair: Prof. Nicholas Bellamy and Prof. Bill Dawson

1705–1730

(32) Arthritic syndromes that defy diagnosis

Prof. George Ehrlich, University of Pennsylvania, Philadelphia, PA, USA

1730–1755

(33) Immunopathogenesis of autoimmune diseases as a basis for therapeutic targets

Prof. Nicola Woodroffe, Sheffield Hallam University, Sheffield, UK

1755–1830

(34) Inflammation in the pathogenesis of Alzheimer's disease

Prof. David Parkinson, Sheffield Hallam University, Sheffield, UK

1900 Reception

1930–2200

CONFERENCE DINNER

Distinguished Guest and After-Dinner Speaker:

Professor George Nuki, Western General Hospital, Edinburgh, UK

Thursday 24 April

Morning Session

SESSION 7: Advances in Inflammation Pharmacology

Chair: Dr. Brian Callingham and Prof. Laurie Prescott

0800–0825

(35) Mechanisms of action of paracetamol and related analgesics

Prof. Garry Graham, University of New South Wales, Sydney, NSW, Australia

0825–0850

(36) Recent advances in the kallikrein–kinin system in inflammatory disorders

Prof. Jagdish Sharma, University of Kuwait, Safat, Kuwait

0850–0915

(37) Novel developments in therapy of inflammatory diseases

Dr. Alan Lewis, Celgene Inc., San Diego, CA, USA

0915–0940

(38) Insight into the molecular basis of selective COX-2 inhibition

Dr. Günter Trummelitz, Boehringer Ingelheim GmbH, Biberach an der Riss, Germany

0940–1005

(39) COX-2-selective agents: the bad and not so bad news

Prof. Ingvar Bjarnason, Guy's, King's and St Thomas' Medical School, London, UK

1005–1030

(40) Cyclo-oxygenase 2 function is essential for bone fracture healing

Prof. Patrick O'Connor, UMDNJ — New Jersey Medical School, Newark, NJ, USA

1030–1100 Coffee/Tea

1100–1125

(41) Age-related decrease in gastroprotection

Dr. Klara Gyires, Semmelweis University, Budapest, Hungary

1125–1150

(42) Direct evidence for the exclusion of desensitization of polymodal afferent nerves to capsaicin in the gastro-intestinal tract of healthy human subjects during two weeks treatment with capsaicin

Prof. Gyula Mózsik, Pécs University of Medicine. Pécs, Hungary

1150–1215

(43) Mechanisms of adverse reactions from NSAIDs and analgesics: approaches for prevention

Prof. Kim Rainsford, Sheffield Hallam University, UK

SESSION 8: Free Oral communications and Poster Presentations

Posters to be erected for entire period of the conference

1215–1345

Poster Discussion: Presenters to give 5–10 minute summary of work on poster followed by 10 minutes discussion

Summing-up and conclusions by Chairpersons

SESSION 1:

Osteoarthritis — Aetiology and Clinical Manifestations

1**Osteoarthritis: symptoms and signs**

WALTER F. KEAN, W. WATSON BUCHANAN and ROBERT KEAN

Musculoskeletal Unit, McMaster University, Hamilton, Ontario, Canada

Osteoarthritis is a loss of the functional and/or biochemical integrity of joints, and is the most common form of arthritis. The disease outcome is variable, but in some individuals it can result in significant dysfunction, disability, socio-economic destruction and sometimes socio-economic failure (Buchanan and Kean, 2002a–d). The main cause of work disability in North America is arthritis: the main cause of disability over age 15 is arthritis; and more than 50 percent of seniors will develop osteoarthritis. There is a poor correlation between clinical features and radiological or other imaging findings. Thus diagnosis and hence correct therapeutic interventions rely heavily on clinical examination findings. The clinical symptoms of osteoarthritis consist of joint stiffness, pain, and dysfunction, but the principal problem for the majority of patients is pain. There are no dedicated pain receptors in cartilage. The origin of the pain is thought to be due to the stimulation of A delta mechanoreceptors and C polymodal nerve endings in the synovium and surrounding joint structures such as the joint capsules, ligaments, and muscles (Buchanan and Kean, 2002a, b). However, some of the pain experienced by patients appears to be sympathetic efferent nerve mediated pain (Forrest, 1992). In the majority of patients with osteoarthritis, focal or scattered sites of inflammation are detected in the synovium, cartilage and sometimes the ligaments and eburnated bone surfaces. The inflammatory process involves a complex series of interacting mechanisms which includes degradation of cartilage by pro-inflammatory cytokines such as IL-1, TNF alpha and IL-6 (Buchanan and Kean, 2002b), and uncontrolled activity of other enzymes which result in the degradation of cartilage and extracellular matrix macromolecules. In addition, the neuropeptide substance P activates inflammatory cells and stimulates the secretion of IL-1 and potentiates the action of this cytokine. IL-1 and TNF alpha increase prostaglandin synthesis by synovial cells, which in turn, is responsible for many of the symptoms identified in patients with osteoarthritis (Buchanan and Kean, 2002b).

The management of osteoarthritis is multidisciplinary and involves collaboration of the health and social disciplines. American and British therapeutic guidelines designed for the safe and effective management of osteoarthritis, advocated initial drug treatment with Acetaminophen, but this strategy has failed to provide adequate therapeutic protection to the patient, since Acetaminophen is ineffective in all but mild cases, and can cause serious gastrointestinal damage, and sometimes liver toxicity, at the full doses recommended. The major therapeutic limitations in the management of osteoarthritis are the side effects of the medications, especially the traditional COX 1 inhibitor non-steroidal anti-inflammatory drugs (NSAIDs)

which produce side effects in up to 30 percent of individuals, especially if exposure is long-term, greater than four weeks. The newer COX 2 inhibitors have lower gastrointestinal toxicity, but come with their own group of potential side effects. The major adverse effects involve the gastrointestinal tract, the renal and cardiovascular homeostatic mechanisms, the liver and the skin. The majority of the side effects are directly attributable to inhibition of prostaglandin synthesis. In addition to social support, education, physiotherapy, occupational therapy and the oral NSAIDs, treatment also includes the use of local or topical NSAIDs, narcotic and non-narcotic analgesics, injection treatments, disease modifying drugs, gene therapy and surgery.

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- Forrest, J. B. (1992). Sympathetic mechanisms in post operative pain. *Can. J. Anaesth.* **39**, 523–527.

2

Cell biology of cartilage in osteoarthritis

LINDA J. SANDELL^{1,2}

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² Department of Cell Biology, Washington University of Medicine, Barnes-Jewish Hospital, St. Louis, MO 63110, USA

Cartilage is comprised of a large amount of functional extracellular matrix that was made and is maintained by a relatively small number of chondrocytes, the sole resident cell type. Normal cartilage exists in a relatively steady state: that is, the anabolic processes, those that result in the synthesis of cartilage matrix components, are in equilibrium with the catabolic processes, those that result in the normal turnover of matrix molecules. If the functional extracellular matrix is disturbed either by physical or molecular means, the cells respond in an attempt to repair the matrix. This stimulation of remodeling and repair is thought to be successful for many years, however, eventually the newly synthesized and activated catabolic enzymes degrade the matrix components. In this presentation, we will propose a new paradigm for understanding OA in the context of cell biology. We have divided the process into three steps: Step 1 is the assault to the cartilage whether by direct impact damage (injury), faulty matrix molecules (genetics) or an unknown stimulus.

Step 2 is the response of chondrocytes to try to repair the extracellular matrix. This attempt to repair sets up a cycle of anabolism and catabolism that eventually results in cartilage erosion. Step 3 is the final descent into cartilage degradation from which there is no recovery. We will examine some very recent evidence that provides a mechanism for this interpretation and possible intervention strategies based on early detection of chondrocyte metabolic activity.

3

Regulation of bone lysis in inflammatory diseases

DAVID HAYNES

University of Adelaide, Adelaide, SA, Australia

Focal bone erosion is a major pathological feature of several common inflammatory diseases such as rheumatoid arthritis and periodontal disease. This pathological bone loss is due to elevated osteoclast resorptive activity in the region. Inflammatory mediators produced by cells in these tissues are thought to cause the elevated osteoclast activity. Over the past decade there have been major advances in our understanding of the factors that regulate osteoclast formation and activity. It is now apparent that receptor activator for $\text{NF}\kappa\beta$ (RANK), its ligand, RANKL (also known as TRANCE, osteoclast differentiation factor and osteoprotegerin (OPG) ligand) and the RANKL inhibitor, OPG, are the major factors regulating osteoclast formation (Lacey *et al.*, 1998; Yasuda *et al.*, 1998; Kong *et al.*, 1999). While much is known about the way these molecules influence normal bone physiology, comparatively little is known about the activity of these molecules in human disease. However, there is now growing evidence that RANK-RANKL interactions also regulate osteoclast formation in disease (Haynes *et al.*, 2001a,b; Crotti *et al.*, 2003). This presentation reviews recent findings comparing expression of RANK, RANKL, OPG and other inflammatory mediators in several human diseases including rheumatoid arthritis, periodontal disease and peri-implant loosening. Immunohistological analysis and *in situ* hybridisation was carried out on frozen human tissue biopsy specimens. The levels of expression of the various factors were evaluated by computer-assisted image analysis and semi-quantitative analysis. The levels of mRNA encoding for RANKL, RANK, OPG, $\text{IL-1}\beta$, $\text{TNF}\alpha$ and factors expressed by mature osteoclasts were measured in the tissues using reverse transcriptase polymerase chain reaction (RT-PCR). In addition, cells were isolated from these tissues and cultured on slices of whale dentine. The formation of large numbers of resorption pits demonstrated elevated numbers of osteoclasts present in the inflammatory tissues near sites of focal bone erosion. Overall, our results showed that there was a both an elevation in the expression of RANKL and a reduction in OPG expression in the tissues adjacent to bone erosion in all the different inflammatory diseases. This shows that OPG and RANKL are likely to be key molecules regulating bone loss in

a variety of diseases and that therapeutic intervention that targets these molecules may be helpful in treating a wide range of bone and joint diseases.

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4

Vascular mechanisms in osteoarthritis and relevance to treatment agents

P. A. CHERAS¹, G. V. L. NIELSEN², E. A. BLACKWELL³,
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² Department of Orthopaedics, Mater Health Services, Brisbane, Qld, Australia

³ Haematology Department, Greenslopes Private Hospital, Brisbane, Qld, Australia

⁴ Department of Orthopaedics, Greenslopes Private Hospital, Brisbane, Qld, Australia

Vascular and haematological factors (balance between coagulation and inflammation) in osteoarthritis (OA) provide a conceptual framework that explains many of the histological, biochemical physiological and clinical aspects of the disease and suggest a role for normalisation of haemostasis in OA treatment (Cheras *et al.*, 1993, 1997; Cheras, 1997; Ghosh and Cheras, 2001).

Aim: To assess the influence of calcium pentosan polysulphate, (CaPPS) and glucosamine hydrochloride/chondroitin sulphate (GHCS) on coagulation, fibrinolysis and clinical outcomes in OA.

Methods: Case control pilot study 1 ($n = 8$): oral CaPPS 10 mg/kg was given twice weekly for 4 weeks, followed by 12 weeks wash out. This scheme was repeated twice. Case control pilot study 2 ($n = 4$): oral GHCS (1.5 g glucosamine HCl and 1.2 g chondroitin sulphate) was given daily for 9 weeks. For study 1 and study 2, blood sampling for measures of procoagulant tendency and fibrinolysis and clinical assessment (WOMAC) were performed at commencement and completion of the treatment periods.

Results: Study 1: Baseline vs. treatment (for first, second and third treatment periods, respectively). PPS reduced procoagulant tendency; platelet aggregation response decreased (80 vs. 73%, 77 vs. 63% and 84 vs. 72%) and Fg fell marginally (2.9 vs. 2.8 g/l, 2.8 vs. 2.7 g/l and 3.1 vs. 2.7 g/l). Fibrinolysis was enhanced; ECLT decreased (190 vs. 175 min, 212 vs. 151 min and 206 vs. 152 min) and PAI-1 activity fell (19 vs. 12.9 IU/ml, 20.6 vs. 18.9 IU/ml and 15.1 vs. 10.8 IU/ml). WOMAC score decreased, consistent with improved clinical outcome (65 vs. 41, 39 vs. 25 and 29 vs. 24).

Study 2: GHCS also decreased procoagulant indicators; platelet aggregation response (83 vs. 73%) and Fg (3.5 vs. 3.0 g/l). Fibrinolysis was enhanced, ECLT normalised (173 vs. 130 min) and $\alpha 2$ antiplasmin fell (91 vs. 75%). Clinical outcome improved, WOMAC 45 vs. 30.

Conclusions: PPS and GHCS decreased procoagulant tendency and improved fibrinolysis coincident with improved WOMAC score, consistent with a role for normalisation of haemostasis in clinical outcomes.

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5

Molecular regulation of chronic inflammation

JACK GAULDIE, MARTIN KOLB, PATRICIA SIME and CARL D. RICHARDS

*Center for Gene Therapeutics, Department of Pathology and Molecular Medicine,
 McMaster University, Hamilton, Ontario, Canada*

To investigate the involvement of a number of cytokines, chemokines and growth factors in the pathogenesis of chronic inflammation and tissue remodeling, we have developed recombinant adenovirus vectors expressing such genes to enable the transfer and transient over-expression of single factors to tissue cells, much as would be the case during prolonged tissue activation or injury. We have transferred many cytokines to tissues such as lung, peritoneum and joints. The results are both expected and surprising, with many genes causing transient pathology but rapid return to normal tissue. A few can initiate a switch to chronic progressive inflammation and tissue remodeling such as fibrosis. Genes such as TGF β can initiate a progressive and chronic fibrogenesis with profound tissue remodeling, including the induction of myofibroblasts. Remarkably, over-expression of TNF α causes pronounced transient inflammation with little resultant tissue damage or lasting effects, while over-expression of IL-1 β causes remarkable tissue damage

and progressive fibrogenesis. Expression of IL-1 β or growth factors such as Oncostatin M within the joint space induces a destructive and progressive arthritis. These systems have helped clarify the central role of molecules such as IL-1 β , TGF β and Oncostatin M in the chronic inflammation that is associated with progressive tissue remodeling seen in fibrosis and arthritis.

6

Experimental pain models — mechanisms of pain

S. D. GAULDIE¹, D. S. MCQUEEN¹, N. CLAYTON² and I. P. CHESSELL²

¹ *Division of Neuroscience, University of Edinburgh, Edinburgh, UK*

² *Neurology CEDD, GlaxoSmithKline, Harlow, UK*

With both rheumatoid arthritis (RA) and osteoarthritis (OA) the primary complaint of patients coming to seek treatment is a chronic and sometimes debilitating joint pain. The majority of treatment regimens are centred around the disease as an inflammatory condition, with the primary focus on reducing the inflammation as a means of treating the associated pain. In many cases traditional anti-inflammatory drugs, while minimising joint inflammation and destruction, do not adequately control the pain experienced by patients. Furthermore, the severity of the arthritis is not always indicated by the patient's level of discomfort. In focusing on the inflammation as the primary concern of arthritis far less attention has been paid to the role of the sensory nerves that innervate the inflamed tissue, transmit the nociceptive signals and, through peptide release from peripheral terminals, may help to perpetuate the disease.

Animal models have provided a large amount of information about the mechanisms underlying joint damage in arthritic conditions such as RA and OA. Although these models do not represent exactly the pathology or aetiology of the human conditions they do provide a platform on which to test biological or pharmacological interventions in the disease process. Using a unilateral model of chronic adjuvant arthritis in both the rat and mouse we examined the role of peripheral nociceptors in the inflammatory process and how the responses of the nerves are altered during chronic inflammation. The unilateral nature of the models allowed the quantification of inflammation and hyperalgesia in the arthritic joint through the ratio of joint diameter and weight distribution in the hind legs, respectively. Using electrophysiological techniques and the close-arterial injection of drugs into the knee joint we were able to examine the plastic changes of peripheral nociceptor responses during inflammation and the underlying pharmacology of these changes.

The responses of peripheral nociceptors to known algogens such as capsaicin, bradykinin, and ATP have been examined in both normal and arthritic animals. Also, we have been able to examine how certain inflammatory mediators change the sensitivity of receptors to these algogens and alter the activation threshold of

the nociceptor. Information gathered from these experiments can draw connections between inflammation and pain, thereby providing targets for therapeutic intervention in the periphery where the painful sensations are initiated.

7

Health status assessment in clinical research and clinical practice environments in osteoarthritis

NICHOLAS BELLAMY

Centre of National Research on Disability and Rehabilitation Medicine (CONROD), The University of Queensland, Mayne Medical School, Herston Road, Brisbane, Qld 4006, Australia

Health status assessment is a key feature of descriptive, prognostic and evaluative clinical research activities. In evaluative research protocols, it is performed to acquire data, the analysis of which will be used to test hypotheses relating to efficacy, effectiveness, safety/tolerability, cost-effectiveness and/or cost-utility. The method of identifying and selecting relevant measures for a clinical trial is driven by a number of factors, including the research question(s), the characteristics of the intervention, regulatory and clinical requirements, and knowledge and experience of available measurement procedures.

Between 1980 and 2002, the bare landscape of clinical metrology in osteoarthritis was transformed from an environment in which outcome measurement was largely ad hoc and poorly standardised, to one in which there is now consensus on core measurement domains and recognition of the value of particular measurement techniques and instruments. This development has largely affected clinical trials environments and is yet to have a major impact on measurement procedures used in routine clinical practice. To be useful, outcome measures require to be valid, reliable and responsive. Their application requires standardisation, and in the case of multi-centre international trials, the cross-cultural adaptation and alternate-language translation of health status questionnaires requires to have been conducted with rigour.

Consensus on outcome measurement domains for future clinical trials in osteoarthritis was reached at the OMERACT III Conference in Cairns, Australia, and was ratified shortly thereafter by the Osteoarthritis Research Society International. Pain, physical function and patient global assessment are the three core set clinical measures for OA clinical trials. The OARSI Guidelines document provides direction with respect to various aspects of the clinical trial, including the selection of specific outcome measurement procedures and instruments.

With respect to efficacy assessment, measurement tools may be divided into three groups: general arthritis measures, disease-specific measures, and generic health-related quality of life measures. The WOMAC Index, Lequesne Indices of Clinical Severity, AUSCAN Index and FIHOA each represent disease-specific indices developed specifically to measure outcomes in osteoarthritis patients. These

instruments differ conceptually and performance-wise and all are valid, reliable and responsive measures of outcome. In contrast, there remains no international consensus on the specific wording and response scale for the patient global assessment question despite its widespread usage. In many clinical trials it may be useful to combine a health related quality of life measure, such as the SF-36 or HUI with a disease-specific measure such as the WOMAC Index or AUSCAN Index.

Most recently the OARSI have developed responder criteria for osteoarthritis clinical trials in hip and knee patients, the proposed criteria having been further refined at the OMERACT VI Conference held in Australia in 2002. Finally, the recently completed ESKE study has quantified the reliability of various examination-based assessment techniques for knee OA, techniques that may find application particularly in trials of purported structure-modifying OA drugs (StMOADs). There are now outstanding opportunities for performing standardised measurements using valid, reliable and responsive instruments and techniques in osteoarthritis clinical trials.

The importation of these techniques, in original or modified form, into routine clinical practice remains challenging, given that the practice requirements of brevity, simplicity and ease of scoring, are compounded by the need to meet resource demands and time requirements in busy practice environments. Three recent surveys in Canada and Australia have suggested that tools routinely used in clinical research are rarely used in clinical practice environments.

The explanation for this lack of utilisation is complex but appears to relate to issues of familiarity, obligation, culture, value and resource requirements. With respect to resource requirements, electronic data capture (EDC) may facilitate the ingress of outcome measurement procedures, currently used in clinical trials, into clinical practice environments. For measurement procedures based on health status questionnaires, evidence to date generally suggests, that EDC is a viable alternative to paper-based data capture, generates comparable information, obviates the need for manual scoring, tabulating and graphing of data and is an effective and efficient method of archiving information.

The introduction of quantitative measurement practices into the routine clinical care of OA patients represents an interesting challenge and may provide an a basis for goal-orientated patient management strategies.

8

The impatient patient: a personal view of osteoarthritis

MICHAEL C. POWANDA

M/P Biomedical Consultants LLC, Mill Valley, CA, USA

Osteoarthritis (OA), especially of the hip and/or knee, is an insidious, decrementally debilitating skeletal muscular disease. The decrease in joint function is generally accompanied by progressively increased pain or at least discomfort and grad-

ually reduced physical, social and work activity. Due to the slowness of onset, the patient often does not realize the true extent of loss of functionality until late in the disease process. The damage that OA does is not necessarily limited to the individual. Families and society as a whole incur the cost of this affliction. In view of the prevalence of OA and its increasing impact on society, OA deserves incomparably more attention from the biomedical community and government funding agencies, especially as a chronic inflammatory disease, such as OA, might affect our susceptibility to other maladies, such as heart disease.

The present options for the treatment of OA may alleviate some of the pain or slow the loss of function, but appear to do little to alter the outcome of disease: inevitable incapacitation or surgical joint replacement. Whilst joint replacement does aid in recovery of function in most cases, the hazards of surgery combined with the present limited duration of the implant makes this option usually one of last resort.

The onset of my OA, in retrospect, was at least 20 years ago and was overlooked not only by myself but also by a number of physicians including at least one rheumatologist. The earliest symptom that I remember was an increased difficulty in climbing over obstacles when starting off on my right side. Both hips became involved; X-rays confirmed minimal joint space consistent with limited range of motion. An exercise program started too late was of little effect. In the end, even more than pain, the driving force for hip surgery was the clearly reduced physical, social and work activity. Ironically, surgery did not rectify this situation as the femoral nerve was damaged during the operation, added evidence that a reluctance to undergo surgery earlier in the disease progress may be justified. However, pain in right knee was virtually eliminated and back pain on the right side reduced. In my experience most analgesics were ineffective at tolerable doses and the pain was more generally felt at the knee or back than at the hip.

My presentation thus will be a reflection on my personal experience with OA, coupled with a plea for more efficacious and safer analgesics, for earlier diagnosis, for earlier therapeutic interventions, perhaps involving physical medicine, micro (nano) surgery or cellular and molecular biology, and for a systems biology approach to prevention as well as treatment.

SESSION 2:

Osteoarthritis — Therapeutics

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Therapy of pain in osteoarthritis — no drugs?

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Three arguments against the use of drugs in osteoarthritis (OA) are advanced:

1. *OA is all about biomechanics.* OA is a disorder of the synovial joints caused by abnormal biomechanics. Damage to the articular cartilage and underlying bone occur at points in the joint where load bearing is maximal. Treatment, therefore, should be aimed at normalising biomechanics.
2. *Joint damage is not the main determinant of pain.* The causes of pain in OA are not understood. The cartilage is largely aneural. Pain may arise from the subchondral bone, and/or from periarticular tissues coming under abnormal mechanical stress as a result of the joint damage. Epidemiological data suggest that the degree of joint damage has little or no influence on the severity of pain, and that other issues, including psychosocial factors, are key determinants of the pain experience. The treatment of pain therefore should target its determinants, not the cartilage or other aspects of the joint (unless new evidence shows that targeting bone, for example, will be successful).

We need to diagnose and treat the different causes of pain in individuals.

3. *People with OA are particularly vulnerable to drug toxicity.* OA is a disorder of older people, associated with many co-morbidities. The toxicity resulting from the inappropriate use of non-steroidal anti-inflammatory drugs in these people is enormous. Drugs should not be given to older people with OA.

Recent developments in pharmaceuticals have transformed some areas of health care. However, it is fallacious to argue that because drugs are helpful in some diseases they should be pursued as the cure for all diseases. OA is a condition that should not be targeted by the pharmaceutical industry. Recent research indicates that both patients and doctors agree with this view-point (Tallon *et al.*, 2000).

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Global treatments for osteoarthritis

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In this forum and others, I have long and often contended that osteoarthritis (OA) is the final common pathway for all manner of insults to the joint, be they traumatic,

inflammatory, congenital, heritable, or structural. One reason for this contention is that OA is generally defined by roentgenographic or pathologic features. But there is considerable variation in presentation: acute inflammatory symmetrical onset in the finger joints of perimenopausal women, usually with a family history thereof, to the isolated asymptomatic presentation in individual joints. Clearly, a variation in treatment reflects not only the severity of symptoms but also whether medical assessment is sought or whether self-medication is used. Many over-the-counter products feature the class label, 'for the minor pains of arthritis', but obviously the person who takes these does not consider the pains minor. Only a large minority of those whose OA becomes symptomatic seek professional care and therefore become patients. For these, a ladder of progressive medications is prescribed, from simple analgesics through anti-inflammatory analgesics in varying dosage to narcotics in a small number of instances. A variety of compounds can be injected into joints. The duration of treatment also varies, prolonged for erosive interphalangeal OA and episodic and of limited duration for brief flares. Traditional medicine, also known as alternative medicine, treats a large number of people; herbal and ayurvedic medicines remain popular in Southern Asia, especially on the Indian subcontinent, and have spread widely throughout the Western world. Acupuncture has made inroads. In more severe cases, surgery offers palliation. Until the process that leads to osteoarthritis can be interdicted, however, no cure is possible.

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NSAIDs and opioids in the treatment of osteoarthritis

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of osteoarthritis (OA). The topical use of these drugs may have the advantage that sufficient local concentration can be achieved also at low serum levels, avoiding general side effects by this way (Vaile and Davies, 1998; Rosenstein, 1999; Heyneman *et al.*, 2002).

The authors try to clarify the following questions:

1. Can be reached sufficient local concentration of the topically administered drug in articular and periarticular tissues?
2. How can be the penetration of the percutaneous drug enhanced?
3. Is the percutaneously administered drug effective compared to placebo or orally administered drug?
4. Are the rate and severity of side effects really smaller?

The answers:

ad 1. On certain conditions satisfactory concentration can be attained by percutaneous administration (Vaile and Davies, 1998; Rosenstein, 1999; Bender *et al.*, 2001; Heyneman *et al.*, 2002).

ad 2. Suitable transport systems, enhancers and/or iontophoresis can considerably increase penetration (Rosenstein, 1999; Heyneman *et al.*, 2002; Paolino *et al.*, 2002).

ad 3. There are data showing that topical NSAID-s are superior compared to placebo, and equal with orally administered drug, although the design of most of the trials is not satisfactory (More *et al.*, 1998; Rosenstein, 1999; Heyneman *et al.*, 2002).

ad 4. The rate and severity of side effects are smaller, but life threatening side effects do occur.

The use of trans-dermal opioids may have advantages: avoidance of gastric intolerance stable blood level, lower rate of side effects (Jeal and Banfield, 1997; Bartleson, 2002). Well-designed RTCs are unfortunately lacking.

Conclusion: In spite of the encouraging results with topical NSAIDs, better designed, multicentre trials, using standard methodology are clearly required. To see the place of percutaneous opioids in the treatment of OA even orientating trials are lacking. Trials including patients with OA and untreatable with other modalities, should be performed.

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12**Meloxicam, a COX-2-selective NSAID: what's a nice drug like you doing in a class like this?**

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From the landmark discovery of the functional activity of the cyclooxygenase (COX) enzyme by Sir John Vane and colleagues in 1971 (Vane, 1971), to the cloning of COX-2 by 3 groups in the early 1990s, the COX field has been an exciting area to work in. Drug discovery was close on the heels of the cloning of the COX-2 enzyme. Phil Needleman and colleagues started the process with discovery

and clinical development of celecoxib; a molecule designed to selectively interfere with the COX-2 unique 'side pocket' (Masferrer *et al.*, 1994). While a number of molecules designed to selectively inhibit the COX-2 enzyme began to enter clinical development, other agents with a better tolerability profile, either on the market or close to approval were being tested to determine whether they selectively inhibited COX-2. One such agent, meloxicam, an NSAID similar in structure to many of the NSAIDs within the oxicam class was shown to possess a markedly benign pre-clinical and early toxicological profile compared to its 'classmates' of the time.

In vitro and *ex vivo* evidence coupled with compelling clinical findings strongly supported the hypothesis that meloxicam selectively inhibits COX-2 (Pairet and Van Ryn, 1999). The large scale MELISSA (Dequeker *et al.*, 1998) and SELECT (Hawkey *et al.*, 1998) trials clearly demonstrated that meloxicam, at doses of 7.5 mg, had a statistically superior and clinically meaningful benefit in terms of GI tolerability over two traditional NSAIDs. Endoscopic studies, while small, coupled with safety data originating from short and longer term trials, suggested as well, that meloxicam appeared to demonstrate a better safety profile when compared to traditional NSAIDs. As such, meloxicam was one of the first agents launched as a drug that could selectively inhibit COX-2.

Continued clinical and pre-clinical data has been collected since the first approval of meloxicam in 1995. To date, well over 100 000 patients have been assessed in a number of clinical trials and regulatory bodies around the world have approved meloxicam for use in patients with osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and other inflammatory conditions. In addition, meloxicam is available in dozens of countries and in excess of 25 million prescriptions for meloxicam have been filled globally. Meloxicam, at doses ranging from 7.5 and 22.5 mg has been shown to be efficacious, safe and well tolerated in a host of patient populations (Degner and Richardson, 2001). As a COX-2-selective agent, meloxicam has been shown to have superior gastrointestinal profile coupled with improved tolerability when compared to more class NSAIDs.

This paper will present further evidence of the improved tolerability, safety, efficacy and effectiveness of meloxicam in patients with osteoarthritis. The effectiveness of meloxicam will be a central focus of the presentation based on data originating from the IMPROVE study recently completed in the United States (Gagnier *et al.*, 2002). While continually debated, evidence from a number of clinical studies strongly supports the statement that meloxicam, while not a coxib, is a COX-2-selective NSAID.

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Dexibuprofen: pharmacology, therapeutic uses and safety

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Nonsteroidal antiinflammatory drugs (NSAIDs), e.g. ibuprofen and S(+)-ibuprofen are effective for the relief of pain and their use is widespread (Evans, 1996; Dionne and McCullagh, 1998; Rainsford, 1999). Ibuprofen, which contains equal quantities of R(–)-ibuprofen and S(+)-ibuprofen, has been used as an anti-inflammatory and analgesic agent for over 30 years. S(+)-Ibuprofen or dexibuprofen (INN) is the single effective and pure enantiomer of ibuprofen. Ibuprofen and INN are differential in their physico-chemical properties (due to different crystal packing, crystal binding energy, e.g. different melting point, heat of fusion and solubility; Leising *et al.*, 1996). Furthermore, the two enantiomers of ibuprofen are different in terms of their pharmacological properties and their metabolic profiles. On the rationale basis that a fraction of the dose of R(–)-ibuprofen undergoes ‘metabolic inversion’ to yield S(+)-ibuprofen, it has been argued that to obtain comparable pharmacodynamic effects a dose of 1 : 0.75 (ibuprofen *vs.* INN) would be needed. On the other hand, inversion is not instantaneous, the extent is variable, depends on dosing situation and is reduced in patients experiencing acute pain.

Several clinical trials and post-marketing surveillance studies were performed to broaden the findings on INN. Attention was directed to dose findings, pharmacokinetics, pharmacodynamics, special indications and safety in humans. Nineteen clinical trials were conducted according to GCP (in operation since 1996) and 12 were earlier clinical trials. In these 31 clinical trials and in 6 post-marketing surveillance studies more than 12 000 patients were treated with INN in a special crystal form.

In the last 5 years 4836 patients have been given INN in clinical trials and post-marketing surveillance trials. Only in 5.2% (252 patients) a total number of 318 adverse events have been reported. Out of these, 249 adverse events were assessed as related to dexibuprofen. The majority of adverse events were gastrointestinal. Moreover, only 3 serious adverse drug reactions (0.06%) were observed. There is clinical evidence that S(+)-ibuprofen provides faster onset and greater analgesia than ibuprofen, but without decreased duration of activity or greater incidence of adverse reactions (Dionne and McCullagh, 1998). In the dose ratio of 1 : 0.5 (ibuprofen *vs.* INN) at least equivalent efficacy was shown in acute mild to severe somatic and visceral pain models like activated osteoarthritis of the hip, osteoarthritis of the knee, rheumatoid arthritis, ankylosic spondylitis, lumbar

vertebral syndrome, distortion of the ankle joint, dysmenorrhoe or postoperative dental pain. INN has been proven to have at least comparable efficacy to diclofenac, naproxen and celecoxib by an favourable tolerability. The cartilage degradation surrogate markers Matrix-metalloproteinases (MMP 1, 3, 8 and 9) are not altered during INN therapy. Furthermore, in a new *in vitro* study it was shown that 100 μ M INN inhibited biosynthesis of LTB₄ by approx. 30%.

These results show, that a dose ratio of 0.5:1 of INN vs. ibuprofen provided adequate analgesia. The results suggest that INN in this special crystal form is a safe and effective treatment for different pain conditions.

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Characteristics of celecoxib prescriptions in the Aquitaine region: data from the regional database of the Sécurité Sociale

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Objectives: To describe the characteristics of subjects beginning a celecoxib treatment and the evolution of the treatment over a six months period.

Methods: All the subjects for which a reimbursement of celecoxib was submitted to the Sécurité Sociale d'Aquitaine between the 1st and the 31st of December 2000, were included in the study. For each subject, analysis of co-prescriptions was done during three periods: the 6 months preceding the date of delivery (anterior period), the day of the delivery and the 30 subsequent days (concomitant period), the 6 months following the end of the concomitant period (posterior period). All the data used for the study were extracted directly from the database, none were obtained by interviews of the included subjects or their practitioner.

Results: A total of 14 323 subjects were included in the study. Their mean age was 61.9 years, 50% were at least 65 years old. The men/women sex ratio was equal to 0.45. Almost two-thirds of the subjects were previous NSAIDS-typical users. During the concomitant period, 15% of subjects had a gastroprotective agent delivery. During the 6 months following the end of the concomittant period, new reimbursements of celecoxib concerned 41% of the subjects, of them 71% had fewer

than 4 reimbursements. Among the subjects who renewed celecoxib, 41% used a gastroprotective agent at least once during the anterior period and 44% at least once during the posterior period. Among the subjects who did not renew celecoxib, these proportions were 32.2% and 33.7%, respectively.

Discussion: These results shows that celecoxib is mainly prescribed to previous typical users of NSAIDs. The low difference of gastroprotective agents prescription between the period preceding and following onset of celecoxib is not in favor of an advantage of celecoxib in terms of lower rate of gastroprotective agents use.

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Assessment of the safety of COX-2 selective drugs

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Despite the undoubted efficacy of non-selective NSAIDs for the control of pain and inflammation in arthritis and musculoskeletal injury, a significant proportion of patients taking these drugs experience gastrointestinal symptoms and lesions, which range from petechial hemorrhages to fatal complications of peptic ulcer.

The risk of developing a severe GI adverse event varies from patient to patient, and from NSAID to NSAID. Epidemiological studies have shown that NSAIDs increase the overall risk of peptic ulcer bleeding (OR 3.09–4.5), adverse events-related hospitalizations (OR 3.9–5.5), GI surgery (OR 7.75), and GI adverse events-related death (OR 4.79–7.62). Factors predisposing NSAID users to greater risk include: age older than 60 years (OR 2.86), previous ulcer history or ulcer bleeding (OR 4.76–9.5), high dose or multiple NSAIDs (OR 4.0–23.3), concomitant corticosteroid therapy (1.83–4.4), and concomitant anticoagulant therapy (OR 2.1–16). A recent UK study indicates that risk of bleeding with <1 defined daily dose of NSAID is associated with a 10-fold risk of bleeding which rises to >20-fold for patients taking >1 defined daily dose. Moreover, *Helicobacter pylori* infection increases the risk of NSAID-associated ulcers and also increases the risk of such ulcers bleeding.

The new drugs, which have been developed to minimize adverse GI effects include the COX-2 selective inhibitors, which effect their benefit through sparing the COX-1 isoform of cyclo-oxygenase.

Extensive safety studies have been undertaken in the development of the coxibs. For rofecoxib and etoricoxib, the evaluations have included studies of ⁵¹Cr-labeled fecal red blood cell loss, macromolecular permeability of the small intestine, upper GI endoscopy studies in arthritis patients and more recently large clinically relevant outcome studies such as the presence of a clinically relevant ulcer(s), perforation and/or bleeding. These studies confirm that patients taking a coxib have a significantly lower rate of gastric or duodenal ulcers and ulcers complicated

by bleeding than do patients taking conventional NSAIDs. The CLASS (Celecoxib Long-term Arthritis Safety Study) and VIGOR (Vioxx Gastrointestinal Outcomes Research Study) studies, each of which enrolled more than 8000 patients, showed an approx. 50% risk reduction for perforations, ulcers and bleeds. The VIGOR study confirms reductions of >50% in clinical upper GI events (54%), complicated upper GI events (57%) and bleeding from anywhere in the GI tract (62%).

In the CLASS study, patients taking aspirin for cardiac prophylaxis in addition to celecoxib did not benefit to the same levels of risk reduction as those who did not require aspirin and took celecoxib alone.

The issue of co-therapy with aspirin is complex but also important due to the widespread use of low dose aspirin (LDA) for secondary prevention of myocardial infarction and thrombotic stroke. LDA is associated with an approx. 2.6-fold increase in bleeding risk which increases in the presence of *H. pylori* infection to approx. 5.6-fold. Moreover, LDA increases the risk of bleeding in patients taking NSAIDs and coxibs and the risk benefit is closely related to the CVS risk.

There was a slight but significantly higher rate of non-fatal myocardial infarction in patients taking rofecoxib (0.4%) compared with those taking naproxen (0.1%) in the VIGOR study. This difference is also consistent across all the comparative trials of rofecoxib with naproxen but is not seen with other non-selective NSAIDs. Naproxen effects an 95% inhibition of thromboxane across the whole dosing interval, which likely provides a protective effect similar to that of aspirin and may be one explanation for these differences. However, COX-2 selective inhibitors also inhibit prostacyclin production in the endothelial cell while sparing COX-1 and normal platelet function. Ongoing studies will address these effects and the difference observed in myocardial infarction rate. In any event, patients with cardiovascular risk factors who are taking a coxib should be given aspirin prophylaxis.

The halving of GI risk seen in the VIGOR study brings the risk of a complicated GI event when taking a coxib close to baseline risk but does not reduce it to zero. There is a background risk of ulcer disease in the population, which was approx. 7% in one coxib study although this has fallen to approx. 1.5% in a more recent study. This fall in placebo ulcer rate may be due to at least two factors: the decline in *Helicobacter pylori* prevalence and the introduction of the coxibs. A significant relative risk reduction of 51% was seen in the VIGOR study in those with ≥ 1 risk factor (RR = 0.49, 95% CI (0.32–0.65)) compared with a reduction of 88% in those with no risk factors (RR = 0.12, 95% CI (0.04–0.98)).

The introduction of COX-2-selective agents offers the opportunity for significantly improved safety for patients who require an NSAID. Although no data is yet available, in patients who are at high risk of an ulcer or complication or those at intermediate risk and requiring an anti-inflammatory drug and low dose aspirin for cardiovascular protection, a PPI should be considered as co-therapy.

SESSION 3:

Osteoarthritis — Emerging Developments

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Current strategies and emerging developments

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The total and relative number of individuals in the population afflicted with osteoarthritis (OA) is increasing in parallel with longevity and epidemic obesity. Although prevalence increases with age, disability often starts when individuals are still pursuing fully active lives. OA is a major burden of disease in our society. The best way to quantify this seems to measure disability-adjusted life years (DALY). One recent study found OA to rank number 5 in women and number 6 in men superseded only by ischemic heart disease, alcohol dependence, diabetes, depression and in men also drug overdose (Kominski *et al.*, 2002).

Progress has been made in recent years regarding risk factors and pathogenesis of osteoarthritis (Nuki, 2002). The new knowledge opens considerable possibilities to transform and improve management of OA. Preventive measures should not only include avoidance of overuse and occupational microtraumata, overweight, smoking (controversial), but also maintenance of good muscle strength. Comfortable shoe wear excluding high heels and other fashion fads perhaps will prevent some knee and hip OA although there is no proof yet. Several recent investigations document low grade inflammation in both early and later stages of OA (Sowers *et al.*, 2002). Crystal deposition may be a common initiating trigger. Inflammatory cytokines, notably IL-1 beta, are certainly involved in the destructive process. This raises the question whether anti-inflammatory pharmacotherapy should be attempted. Perhaps locally delivered IL-1Ra or one of the milder DMARDs used in the treatment of rheumatoid arthritis would be beneficial.

There is ample evidence that fragments from extra cellular matrix act as mediators of catabolic events in OA cartilage (Homandberg *et al.*, 1998). This raises two questions: is this vicious circle a possible target for therapy, and is there an early reversible stage of OA before the matrix fragments are at work and which could be targeted? This leads to the important issue of identifying early OA in the population. Ongoing projects, both retrospective (Lachance *et al.*, 2002) and in particular the prospective Spensult cohort project in Sweden (Petersson *et al.*, 1997), will no doubt yield interesting results and leads for improved management. This study identified some 200 representative individuals aged 35 to 55 in a population and follows them with clinical and laboratory measures as well as imaging techniques.

Finally better insight in pain mechanisms and pharmacological pain control will help to improve life quality of OA sufferers. The search for better and safer pain controlling remedies will continue, driven by scientific curiosity, patient needs as well as market forces. However, despite the fact that my own trade has been that of an rheumatologist/internist, I have to admit, that for times that we can oversee, total

joint replacement remains the major tool with beneficial effect in advanced large joint OA. But the efforts to prevent the occurrence and progression of destructive OA and thereby reduce the need for orthopaedic intervention has high priority.

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Phase-III results of licofelone (ML3000), an inhibitor of COX-1, COX-2 and 5-LOX, in osteoarthritis and endoscopic studies

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Background: Licofelone (ML3000) ist the first in a new class of drugs that suppresses inflammation and pain by competitively inhibiting three enzymes: cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase (5-LOX). There is increasing evidence that NSAID-induced GI toxicity may involve alternative processing of arachidonic acid by 5-LOX to form leukotrienes. It was postulated that inhibition of leukotriene formation by an inhibitor of both 5-LOX and both COX isoenzymes can provide anti-inflammatory effects without GI toxicity and without the negative effects of selective COX-2 inhibition. As leukotriene-modulated mechanisms are involved in the pathogenesis of osteoarthritis (OA) licofelone may offer additional benefits for the treatment of OA.

Objectives and methods: The innovative mechanism of action and the anti-inflammatory and analgesic efficacy of licofelone was shown in a full series of preclinical studies. The GI, renal, hepatic and general tolerability was excellent. In clinical phase II and phase III studies in patients with osteoarthritis the efficacy/tolerability profile was studied in comparison with placebo and active treatment. In an endoscopic study, 121 subjects with normal gastric and duodenal mucosa were treated for 4 weeks with licofelone 200 mg bid or 400 mg bid, placebo or naproxen 500 mg bid. The mucosa was evaluated with modified Lanza scores. Ulcers (3 mm or more in diameter) were assessed. In another study, 148 patients with OA were treated for 12 weeks with licofelone 200 mg bid or naproxen 500 mg bid. Efficacy was evaluated using the WOMAC index. Responders were defined to show a 30% improvement versus baseline.

Results: In the endoscopic study, the gastric mucosa was completely normal after 4 weeks in 93% (200 mg bid), 89% (400 mg bid), 90% (placebo) and 37% (naproxen) of subjects. No ulcers were present in either licofelone group or the placebo group. In the naproxen group 6 ulcers (20%) were observed (5GU, 1 DU). In the 12-week study the efficacy of licofelone 200 mg bid was comparable or slightly better than in the naproxen group. With licofelone, the mean WOMAC index was improved by 23.3 mm (VAS) and with naproxen there was an improvement by 21.5 mm. For the WOMAC index, 69.4% of licofelone-treated patients were responders (68.4% with naproxen). GI adverse events were reported by 13.9% (licofelone) and by 26.3% (naproxen) of patients.

Conclusion: Treatment of OA patients with licofelone was shown to have a comparable efficacy as conventional NSAIDs but to have an excellent GI and general tolerability. The combined inhibition of both COX-isoenzymes and of 5LOX avoids the obvious disadvantages of selective COX-2 inhibitors (e.g. thromboembolic risk) but spares also the GI mucosa. Phase III clinical studies are already completed. The innovative mechanism of action may offer a new alternative for the treatment of OA with an optimal benefit/risk ratio.

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Percutaneous analgesics and NSAIDs in osteoarthritis

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Topical analgesics and NSAIDs are widely used topically to treat local inflammatory conditions. The therapeutic efficacy, as distinct from their placebo effect, of such agents has been controversial. Recent biopsy and micro-dialysis data in rats and in humans have suggested that the depth of penetration may be limited after topical application and that at later times, delivery via the systemic circulation dominates. Both the formulation and nature of the agent used can affect their delivery through the skin. The nature of the agent and local blood flow can also affect its delivery to deeper tissues. Agent potency is also a determinant of the therapeutic effect. It has been suggested that the frequent use of topical NSAIDs for osteoarthritis may be more based on a supportive therapeutic effect than on their efficacy. Whilst large double-blind RCTs quantifying the benefits of topical NSAIDs are lacking, some trials have shown efficacy. Recent developments have occurred in topical drug delivery systems (microneedles, iontophoresis, sonophoresis, micro-emulsions, etc.) are likely to be more widely used in the topical delivery of analgesics and NSAIDs. Most topical agents have no serious side effects but discomfort such as stinging or burning is reported for capsaicin cream and methyl-salicylate is well known to have a rubefacient effect. Cutaneous adverse effects have been reported in about 10–15% of patients using topical NSAIDs.

19**Pharmacology and therapeutics of drugs used to treat osteoarthritis in veterinary practice****P. LEES***Department of Veterinary Basic Sciences, The Royal Veterinary College, London University, Hawkshead Campus, Hatfield, Herts., AL9 7TA, UK*

Pain in animals is not always easily recognised and assessment of the degree of pain can be particularly difficult. Nevertheless, over the last two decades it has been increasingly recognized that animals ‘feel’ pain and almost certainly ‘suffer’ pain as do humans and both subjective (e.g. visual analogue scales) and objective (e.g. force plate) indices of pain have been used (Lipscomb *et al.*, 2002). Therefore, there is now widespread use of analgesic drugs in all major veterinary species. Thus, on welfare grounds analgesic drugs are now used frequently (a) perioperatively, (b) to treat acute trauma (e.g. road accident cases) and (c) to treat chronic pain associated with musculo-skeletal conditions. The commonest cause of chronic pain in dogs and horses is that associated with arthritic conditions, osteoarthritis (OA).

For companion animal species (dog, cat, horse) the drug classes used most frequently to relieve pain and/or suppress inflammation in conditions such as OA are glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and agents described as disease modifying/chondroprotective, including hyaluronan, pentosan polysulphate and polysulphated glycosaminoglycan (PSGAG) (Lees *et al.*, 1999). Corticosteroids, hyaluronan and PSGAG are available in products with veterinary marketing authorizations for intravenous and/or intramuscular and/or local (intra-articular) administration. In addition, in recent years, there has been widespread use in canine and equine medicine of agents described as nutraceuticals. These products which usually contain glucosamine and/or chondroitin have not been well researched for bioavailability and clinical efficacy in the past, but data is now accumulating to suggest that they may have a place in veterinary therapy in the treatment of OA.

Recommendations for management of veterinary subjects with OA are both therapeutic and non-therapeutic. A key non-therapeutic consideration, particularly in the dog, is control of body weight. A further consideration is exercise level, with most clinicians now recommending moderate exercise to maintain joint mobility. However, therapeutic intervention is also necessary and the drugs used most widely are those of the NSAID class, including phenylbutazone, meclofenamate, tolfenamic acid, flunixin, meloxicam, eltenac, vedaprofen, ketoprofen and carprofen. Studies in the author’s laboratory have established the basic pharmacokinetic profiles of most of these drugs in several companion and farm animal species. The key characteristics are moderate or good bioavailability, a high degree of protein binding, and low volume of distribution after parenteral and oral dosing in all species. However, there are major species differences in clearance and half-life (up to 1000%)

which do not follow any predictable pattern. A further point of interest is the fact that the three 2-arylpropionate NSAIDs carprofen, ketoprofen and vedaprofen, are chiral compounds licensed as racemic mixtures and all show marked enantioselectivity in their pharmacokinetics (including in some instances chiral inversion) and pharmacodynamics (Landoni *et al.*, 1997).

NSAID pharmacodynamics have been studied in the author's laboratory using tissue cage models of acute inflammation in dogs, horses and several farm animal species. These have allowed *ex vivo/in vivo* determination of time course and magnitude of inhibition of the cyclo-oxygenase isoforms COX-1 and COX-2 by therapeutic doses of NSAIDs. The data obtained suggests that in most species the compounds listed in the previous paragraphs are non-selective inhibitors of COX-1 and COX-2 although vedaprofen may be COX-1 selective in the horse and carprofen may be COX-1 selective in man, non-selective in the horse, COX-2 preferential in the dog and COX-2 selective in the cat. From whole animal studies using the tissue cage model it has been possible to calculate for several NSAIDs pharmacodynamic constants including E_{\max} , EC_{50} , $T_{1/2KE0}$ and slopes of the concentration effect relationship. In other studies PK-PD integration and the derivation of pharmacodynamic parameters has been undertaken using clinical endpoints such as stride length, skin temperature, swelling etc. (Toutain *et al.*, 2001). These PK/PD modelling approaches at both molecular and clinical levels have advantages over older conventional dose determination studies, in that they allow quantification of (a) the entire concentration-effect relationship, (b) the time-course of that relationship and (c) sensitivity as reflected in slope. From these data it is possible to make rational recommendations on dosage for clinical use on a species by species basis.

These whole animal studies have been supported by *in vitro* studies of COX-1 and COX-2 inhibition by NSAIDs using whole blood assays. These have revealed some species differences both in potency of inhibition of COX isoenzymes and COX-1 : COX-2 inhibition ratios. We now recommend that in place of the more conventional COX-1 : COX-2 ratio based on EC_{50} values, it is more rational to determine for example the IC_{10} COX-1 : IC_{90} COX-2 ratio. This approach will enable the selectivity of newer selective COX-2 inhibitors, which are certain to be licensed for veterinary use in the foreseeable future, to be determined. In fact, one such drug, deracoxib, has been licensed for OA therapy in the dog in the USA, but not in Europe (Millis *et al.*, 2002). A further development has been the recent licensing of tepoxalin, a dual COX/5-LO inhibitor for treatment of canine OA.

There is also considerable veterinary interest in both the nature and mechanisms of action on cartilage biology of drugs used to treat OA. For example, PSGAG, hyaluronan and carprofen stimulate the synthesis of cartilage matrix by equine chondrocytes in culture and, to a lesser degree, by equine cartilage explants also in culture (Armstrong and Lees, 1999). Whether such *in vitro* actions have significance for clinical subjects with OA is less clear, but it is of interest to note that a disease modifying action of carprofen (reduced progression of morphological changes in

cartilage and subchondral bone) has been demonstrated in the dog in a model of OA (Pelletier *et al.*, 2000).

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Inhibition of clinical benefits of aspirin on first MI by non-steroidal anti-inflammatory drugs

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Context: In numerous randomised trials of secondary and primary prevention and their meta-analyses, aspirin reduces risks of recurrent and first myocardial infarction (MI). Recent data provide plausible mechanisms and a suggestion in secondary prevention concerning the possibility that non-steroidal anti-inflammatory drugs (NSAIDs) may interfere with the clinical benefits of aspirin.

Objective: to evaluate whether NSAIDs inhibit the clinical benefit of aspirin of first MI.

Design and setting: Subgroup analysis from a randomised double-blind, placebo controlled trial of 325 mg aspirin on alternate days with 5 years of follow-up with prospective observational data on use of NSAIDs.

Setting and participants: 22 071 apparently healthy US male physicians.

Main outcome measures: Self-reported, first MI confirmed by reviews of medical records.

Results: A total of 378 MIs were confirmed. Among participants randomized to aspirin, there was 44% reduction in the risk of MI compared with those who were randomized to placebo. This beneficial effect was attenuated among those who use NSAIDs chronically (≥ 60 days per year). Intermittent use (less ≤ 60 days/year) did not materially alter the benefit of aspirin.

Conclusions: These data suggest that regular but not intermittent use of NSAIDs inhibit the clinical benefits of aspirin. Chance, bias and confounding remain plausible alternative explanations, despite the prospective design and adjustment for a large number of covariates. Nonetheless, we believe the most plausible interpretation of the data to be that NSAIDs inhibit the clinical benefit of aspirin on first MI.

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The cardiovascular effects of concurrent non-selective anti-inflammatory drugs and aspirinMARIE HUDSON¹, MURRAY BARON², ELHAM RAHME
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Background: The cardiovascular risks and benefits of non-selective anti-inflammatory drugs (NSAIDs) when used in combination with aspirin (ASA) are unknown.

Methods: This study is a population-based, retrospective cohort study. Using governmental databases, we identified patients who were ≥ 66 years of age, were hospitalized for an index acute myocardial infarction (AMI) between January 1, 1992 and March 31, 1999 and filed a prescription for ASA within 30 days of discharge. The main exposure was the prolonged use of any NSAID, defined as the use of ≥ 30 days of any NSAID, after the index AMI. Other exposure groups were ever use of any NSAID and chronic use, defined as the use of ≥ 60 days of any NSAID, after the index AMI. We also defined subgroups of exposure for prolonged, ever or chronic use of diclofenac, naproxen and ibuprofen. The outcomes consisted of recurrent AMI and death combined and of recurrent AMI alone. Subjects were followed up to one year after the index AMI.

Results: We identified 16 808 patients who met the study entry criteria. Of these, 21.4% received at least one prescription for an NSAID during the follow up period, 3.7% had a recurrent AMI and an additional 5.4% died. The rate of recurrent AMI and death combined was lower in subjects exposed to any NSAID (HR 0.91, 95% CI 0.78–1.07) and, in particular, in subjects exposed to diclofenac (HR 0.75, 95% CI 0.57–0.99), compared to non-exposed subjects. The rate of recurrent AMI and death combined and of recurrent AMI alone in the various groups of exposure are presented in Table 1.

Table 1.

Adjusted hazard ratios of subjects exposed to NSAIDs compared to non-exposed subjects (95% CI in parentheses)

	Any NSAID	Diclofenac	Naproxen	Ibuprofen
Recurrent AMI and death				
Prolonged use	0.91 (0.78–1.07)	0.75 (0.57–0.99)	0.95 (0.70–1.28)	0.75 (0.41–1.36)
Ever use	0.97 (0.87–1.09)	0.85 (0.71–1.03)	1.00 (0.82–1.22)	0.79 (0.54–1.15)
Chronic use	0.92 (0.75–1.13)	0.75 (0.52–1.09)	0.97 (0.64–1.47)	0.84 (0.38–1.88)
Recurrent AMI				
Prolonged use	1.16 (0.90–1.48)	1.02 (0.67–1.55)	1.13 (0.70–1.81)	1.43 (0.67–3.02)
Ever use	1.10 (0.91–1.35)	0.97 (0.71–1.33)	1.07 (0.76–1.50)	1.09 (0.62–1.94)
Chronic use	1.15 (0.83–1.60)	1.10 (0.64–1.88)	0.99 (0.49–2.00)	2.10 (0.86–5.11)

Conclusions: NSAIDs, in particular diclofenac, may add to the cardioprotection afforded by ASA in patients with known heart disease.

Acknowledgements

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SESSION 4:

Osteoarthritis — New Agents and Procedures

22

Inhibitors of iNOS: potential for symptomatic relief and disease modification in osteoarthritis

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The formation of nitric oxide (NO) by constitutive enzymes plays an important role in normal physiology, however, the excessive production of NO by inducible NO synthase (iNOS) has been implicated in the inflammatory process. NO and reactive products, such as peroxynitrite (ONOO⁻), are cytotoxic and produce loss of cell function. There is considerable evidence that excessive NO production from iNOS plays a major role in chronic inflammatory disease such as RA and more recently the activity of iNOS has also been linked to the cartilage destruction in osteoarthritis (OA). In addition to the substantial body of evidence in the literature, the structurally novel selective iNOS inhibitor, AR-C102222 (Tinker *et al.*, 2003), has been used to investigate the role of iNOS in models of both pain and inflammation, as an indicator for the symptoms of OA and also in cartilage degradation, as a measure of the potential for disease modification in OA.

Several studies have shown that NOS inhibitors are anti-inflammatory. In our own studies, the selective iNOS inhibitor AR-C102222 substantially reduced acute oedema and suppressed both inflammatory and neuropathic pain in experimental models. Inhibitors of NOS also have efficacy in chronic models of RA and this efficacy has been confirmed using AR-C102222 (Boughton-Smith *et al.*, 2002). This compound not only reduced the inflammation but also abrogated joint destruction in models of RA. These results suggest that inhibition of iNOS will reduce both the symptoms and potentially reduce structural damage in RA. There is also a substantial body of evidence that iNOS has an important pathological role in OA. In OA there are increases in NO formation and iNOS activity in the cartilage and synovium. The inflammatory cytokines that drive induction of iNOS are also expressed in OA. Further evidence for a role of iNOS in cartilage degradation in OA comes from in-vitro studies using chondrocyte and cartilage in which NOS inhibitors reduce both IL- β induced suppression of matrix formation and activation of matrix metalloproteinases. The formation of NO from iNOS may also be a key mechanism that leads to loss of sensitivity to the growth factor IGF-1 by OA chondrocyte. Non-selective inhibitors of NOS, and the selective iNOS inhibitor AR-C102222, reverse the insensitivity to IGF-1 in OA cartilage and thereby facilitate matrix production. *In vivo* studies, using iNOS^{-/-} animals or an inhibitor of NOS in models of OA have shown beneficial activity on cartilage destruction providing further evidence for an important role of iNOS in the processes underlying OA pathology.

The anti-inflammatory and analgaesic activity of NOS inhibitors, including AR-C102222, suggest that a selective iNOS inhibitor will produce symptomatic relief in RA and OA. The activity of iNOS in OA chondrocytes and the beneficial

effects of NOS inhibitors on chondrocyte function, cartilage degradation and in animal models of OA also provide evidence that selective iNOS inhibitors will suppress cartilage degradation and modify structural changes in OA.

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Nitric oxide, inflammation and NSAID-induced gut injury

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Nitric oxide (NO) is involved in the physiological regulation of gut function, including actions on blood flow and leukocyte adhesion in the microcirculation, as well as the modulation of mucus and alkaline secretion in the stomach (Moilanen *et al.*, 1999). Such beneficial actions of NO can be mimicked by NO-donors and other agents that can generate NO locally, and prevent gut injury. The NO-NSAIDs, agents combining an NO donating moiety with an NSAID, likewise have a beneficial profile in that they have a marked reduction in their propensity to cause mucosal injury and erosion in the gut in experimental models (Wallace *et al.*, 1994).

However, NO, being a reactive radical, can also exert detrimental actions. The role of NO, derived from the inducible isoform of NO synthase, iNOS, in inflammatory bowel disease is controversial, with divergent findings reported with different models, iNOS inhibitors and different gene-deleted animals. Once expressed, iNOS has the ability to produce sustained and substantial amounts of NO, but this appears to be injurious only under appropriate local environmental conditions, when it can provoke tissue injury and inflammation. Thus, NO combines with superoxide to form the highly cytotoxic peroxynitrite, which can subsequently release the hydroxyl radical. The role of these oxygen moieties can be explored *in vivo* using a long-acting pegylated form of superoxide dismutase, SOD-PEG which prevents the microvascular leakage and the epithelial damage in the gut provoked by iNOS expression following lipopolysaccharide (LPS) challenge (Lamarque *et al.*, 2000; Kiss *et al.*, 2001).

Apart from causing acute gastric mucosal injury, NSAIDs such as indomethacin, flurbiprofen and diclofenac provoke an enteropathy some 24–72 h following a single administration in the rat. Jejunal injury and elevation in myeloperoxidase (MPO) are accompanied by site-specific iNOS expression and microvascular leakage. The findings with SOD-PEG and iNOS inhibitors indicate a role for both superoxide and NO in this jejunal injury involving enteric bacteria and LPS release (Evans and

Whittle, 2001). These effects, which appear to reflect peroxynitrite production, thus contrast with the protective actions of NO-donors on NSAID-induced gut injury.

Recent studies have shown that multiple dose of indomethacin can produce a widespread systemic inflammatory response syndrome (SIRS) in the rat, with microvascular leakage and iNOS activity being detected in the lung, liver, spleen and kidney as well as different gut regions, after 48 h. This is inhibited by treatment with antibacterials or polymixin B or by the highly selective iNOS inhibitor GW273629, indicating a role for translocation of gut bacteria and LPS generation with widespread iNOS expression in this gut-origin model of SIRS (Evans and Whittle, 2003).

The present work thus identifies an involvement of both superoxide and NO in a number of models of gut epithelial and microvascular injury, where iNOS is expressed and selective iNOS inhibitors show efficacy. These findings thus implicate a role for peroxynitrite, rather than NO itself, as the injurious mediator *in vivo*. Agents that modify iNOS activity, or remove oxygen radicals including superoxide or peroxynitrite may thus be of value in the therapy of inflammatory gut diseases. Moreover, the findings imply that NO-donating compounds such as the NO-NSAIDs will only exhibit the beneficial and protective actions of NO.

Acknowledgements

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New COX-2-selective CINODs

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Conventional non-steroidal anti-inflammatory drugs (NSAIDs) inhibit both cyclooxygenase (COX) isozymes, COX-1 and COX-2. NSAID gastrointestinal toxicity is largely ascribed to inhibition of physiological prostanoid production by constitutive COX-1. COX-2 selective inhibitors show improved gastric safety over conventional NSAIDs, yet their gastrointestinal and cardiovascular risk profiles pro-

vide further opportunity for clinical improvement. Nitric oxide (NO) is an endogenous gastroprotective, anti-platelet biomediator. We have utilized our novel technology platform to generate bi-functional molecules that incorporate covalently bound NO-donating groups within COX-2 selective inhibitors. For example, isoxazole-benzensulfonamides were synthesized bearing either an alcohol (NMI-1089) or nitrate (NMI-1093) functionality. Both NMI-1089 and NMI-1093 (100 μ M) inhibited isolated human COX-2, but not ovine COX-1. Their *in vitro* COX-2 inhibitory potency relative to celecoxib and rofecoxib was: celecoxib > NMI-1093 > rofecoxib > NMI-1089. NMI-1089 and NMI-1093 were likewise COX-2-selective inhibitors in human whole blood. As COX-2-selective inhibitors, both NMI-1089 and NMI-1093 suppressed prostaglandin E₂ formation and cell infiltration in the rat air pouch inflammation model. By virtue of its NO-donor group, NMI-1093 enhanced gastric ulcer healing *in vivo* and inhibited ADP-induced platelet aggregation *in vitro* and *ex vivo*, in contrast to the non-nitrated COX-2-selective inhibitors. These results demonstrate that the addition of NO-donating groups onto COX-2-selective inhibitors generated new chemical entities endowed with anti-platelet (systemic) and gastric-healing (local) properties that would be expected to improve the therapeutic and clinical profiles of the COX-2 inhibitor drug class.

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A new paradigm in pain and inflammation treatment: COX-inhibiting nitric oxide donator (CINOD)

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Background: The clinical utility of NSAIDs for pain relief is tempered by their propensity to cause gastrointestinal (GI) toxicity. COX-inhibiting nitric oxide donators (CINODs) are a new class of drugs designed to provide analgesic efficacy through balanced COX inhibition and improved GI safety through the protective effects of controlled nitric oxide donation. AZD3582 [4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2 naphthyl)propanoate] is the first CINOD to enter development for the treatment of acute and chronic pain.

Objectives: To evaluate the CINOD paradigm and the potential clinical utility of this new class by reviewing existing evidence from a range of preclinical studies that assessed the pharmacology, efficacy and GI safety of AZD3582.

Methods: Anti-inflammatory and analgesic efficacy were evaluated in rat carrageenan models of pain and inflammation. COX-1 inhibition was evaluated after oral administration of AZD3582 and naproxen in the rat. The effects of AZD3582 on leukocyte infiltration and its inhibition of both COX-1 and COX-2 were evalu-

ated in male Wistar rats using an air-pouch model. The pathway for nitric oxide release was investigated in LLC-PKI epithelial cells in vitro. GI effects were evaluated in comparison with those of naproxen in rat models of acute gastric damage, small-intestinal injury and gastric mucus production.

Results: AZD3582 caused dose-dependent reductions in carrageenan-induced pain and inflammation in rats. Therapeutic doses of AZD3582 and naproxen similarly and extensively inhibited COX-1 in rat whole blood. In the air-pouch model, it also effectively inhibited COX-1 and COX-2. In the same model, AZD3582 reduced leukocyte infiltration in response to carrageenan injection. AZD3582 produced a concentration-dependent increase in cGMP levels in vitro. The cGMP increase was abrogated by the nitric oxide scavenger, haemoglobin, indicating involvement of nitric oxide. The bioactivation pathways involving cytochrome P-450-dependent catalysis may be responsible for nitric oxide release from AZD3582. In rats, AZD3582 was associated with a lower incidence of GI damage than usually observed with naproxen. AZD3582 reduced the extent and severity of gastric damage and did not affect mucus production. Naproxen caused more irritative and corrosive effects (superficial erosions, irregular mucin production and mucosal inflammation) on the gastric and intestinal mucosa than AZD3582. The effects of naproxen were particularly noticeable in the stomach and proximal part of the intestine.

Summary: Preclinical studies demonstrate that the efficacy and potency of AZD3582 as an analgesic and anti-inflammatory agent are similar to those of naproxen. In a series of well-accepted preclinical models, AZD3582 has low ulcerogenic potential and favourable GI safety profile compared with naproxen. AZD3582 inhibited both COX-1 and COX-2, and donated nitric oxide in vitro.

Conclusions: These preclinical studies support the concept that CINODs can provide analgesic and anti-inflammatory efficacy through COX inhibition, and can potentially reduce the incidence of GI damage through the local and systemic effects of nitric oxide donation.

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The CINOD AZD3582 exhibits improved gastrointestinal safety compared naproxen in healthy volunteers

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Background: AZD3582 [4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2 naphthyl) propanoate] is the first COX-inhibiting nitric oxide donator (CINOD) to be extensively

studied in humans. AZD3582 is being investigated as an anti-inflammatory and analgesic agent for the treatment of acute and chronic pain. It has been shown to be effective in animal models of pain and inflammation, and to have a good gastrointestinal (GI) safety profile in rats. AZD3582 has the potential to provide an improved therapeutic index of efficacy and GI safety, through both the inhibition of COX-1 and COX-2, and the tissue-protective effects of nitric oxide.

Methods: The GI safety profile of naproxen vs. AZD3582 and placebo was compared in 31 healthy volunteers. Subjects received naproxen (500 mg b.i.d.), an equimolar dose of AZD3582 (750 mg b.i.d.) and placebo in a double-blind, randomized, 3-way cross-over study over 12 days with a 14-day washout period between each treatment. At the beginning and end of each treatment period, unsedated endoscopy was performed and gastroduodenal damage assessed by the Lanza score and erosion/ulcer counts. Small bowel permeability was determined by differential urinary excretion of lactulose and rhamnose. Pharmacokinetic properties were assessed at steady state. Using a rat air-pouch model, inhibition of COX-1 (measured by PGE₂ concentrations in exudate samples) and of COX-2 (measured by whole blood TXB₂ synthesis) by AZD3582 (3–30 μ mol/kg) was assessed. COX-1 inhibition in rat blood was evaluated after oral administration of AZD3582 and naproxen (0–20 μ mol/kg).

Results: Twenty-seven subjects completed all 3 treatment periods. Gastroduodenal endoscopy, as scored by the Lanza method, showed no erosions with placebo (mean 0; range 0–0) and a reduction ($P < 0.01$) in erosions with AZD3582 (mean 0; range 0–2) compared with naproxen (mean 2; range 2–3). There were no ulcers in the AZD3582 treatment group. There were no changes in intestinal permeability during placebo or AZD3582 treatment, but naproxen increased permeability significantly ($P < 0.05$) (lactulose:rhamnose ratio increased from 0.030 to 0.040). The t_{\max} of naproxen was longer following administration of AZD3582 compared with naproxen (2.7 ± 1.2 h vs. 1.9 ± 0.62 h). AZD3582 provided similar bioavailability (95%) compared with an equimolar dose of naproxen. Therapeutic doses of AZD3582 and naproxen similarly and extensively inhibited COX-1 in rat blood. AZD3582 effectively inhibited COX-1 and COX-2 in the rat air-pouch model.

Conclusions: AZD3582 is rapidly and well absorbed in humans, and in healthy volunteers caused significantly less acute GI toxicity than equimolar doses of naproxen. In rats, AZD3582 inhibited COX-1 and COX-2, suggesting that protection of the GI mucosa is provided by nitric oxide donation. In man, AZD3582 is expected to show similar COX-inhibitory activity to naproxen. These data on GI safety warrant long-term tolerability studies along with anti-inflammatory and analgesic evaluation of AZD3582 in humans.

SESSION 5:

Osteoarthritis: Newly-emerging Therapies

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**Heat-shock proteins and their role in chondrocyte protection,
an application for autologous transplantation**

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Articular cartilage injury presents a unique therapeutic challenge. As cartilage possesses no blood or nerve supply of its own it has a particular susceptibility to early injury and a poor capacity for self repair. Current treatment options are limited and injury can eventually lead to osteoarthritis. Autologous chondrocyte transplantation is an exciting therapeutic development (Peterson *et al.*, 2000), but despite initial encouraging results a number of problems have been identified, most notably graft failure and poor formation of fibro-cartilage as opposed to healthy normal hyaline cartilage. Bleeding is an inevitable consequence of surgery and unfortunately, blood induced cartilage may result (Roosendaal *et al.*, 1997, 1999). It is hypothesised here that protecting chondrocytes against blood could significantly improve results.

Heat-shock protein induction may confer just such a chondroprotective mechanism since they protect cells against usually lethal stressors. This work aimed to determine whether heat shock proteins could be induced in chondrocytes, and whether such an induction is chondroprotective against blood-induced damage. The expression of heat shock proteins in human chondrocytes and rat femoral head cartilage following heat shock was analysed by Western blotting, and red blood cell-induced chondrocyte death was assessed by flow cytometry in the presence and absence of pre-treatment with heat shock. Despite a small sample number we were able to demonstrate heat shock induced expression of heat shock protein 70 (HSP-70) and HSP-32. The induction of HSPs appeared to reduce chondrocyte death in the absence or presence of varying numbers of red blood cells or red cell products. However, as the red blood cell number rose, the damaging effects of blood appeared to outweigh any chondroprotective effect of HSP induction. Chemical induction of HSP-32 appeared to be more effective and may hold greater promise. Novel inducers of the HSP response may provide more efficient and potent protection. These first results need to be consolidated and investigated further.

The induction and chondroprotective effects of heat shock proteins could be applied to increase the initial success of implanted chondrocytes improving the outcome of autologous chondrocyte transplantation.

Acknowledgements

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Combination therapy for inflammation: synergism of NSAIDs/steroids with some herbal/animal products

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Aim: To validate combinations of anti-inflammatory prescription/OTC drugs (AIDs) with some traditional remedies derived from foodstuffs by pharmacological assays in rats with experimental arthritis or fibrosis.

Methods: Polyarthritis was engendered in rats with either (a) mycobacterial adjuvant or (b) collagen type-II plus incomplete adjuvant. Fibrotic inflammation was initiated by injecting zymosan or hydroxyapatite (bone mineral) in a rear paw. Traditional remedies (TR) were emu fat (Aboriginal), celery seed (Ayurvedic), greenshell mussel (Maori), sea cucumber (Malaysian) as dried powders, natural oils or (lipid) extracts prepared with ethanol or liquefied CO₂. Arthritis-ablating potential was assessed by co-administration with arthritogens. Otherwise, treatments were given orally and stomachs examined for haemorrhagic bleeding in rats given NSAIDs or prednisone.

Results: Beneficial combinations were observed at three levels:

1. Amplification of AID potency, when administered to rats developing arthritis.
2. Extension of therapeutic spectrum, indicated by efficacy of AIDs plus TR for treating/remitting NSAID-resistant established arthritis or induced fibrosis.
3. Protecting gastric mucosae from NSAIDs/prednisone and reducing steroid-induced thymus involution.

Active lipid extracts are Celer-ex™, Dromaiol™(emu), Lyprinol® (NZ mussel), and Rhodol (sea cucumber) contained lipoxigenase inhibitors, other antioxidants, gastroprotectants and some unique antimetabolites.

Conclusion: With adequate quality controls for natural products (source, processing, efficacy), this type of integrated medicine = Orthodox & Traditional provide (i) gastro-irritant plus gastro-protective pharmaca, (ii) COX-inhibition plus LOX-inhibition and (iii) analgesic plus arthritis-ablating activities not attainable with monopharmacy at relatively low cost.

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The effects of opioids and cannabinoids on chondrocytes

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Opioid peptides are found in the synovial fluids of patients with rheumatoid arthritis and osteoarthritis where they may have anti-inflammatory effects in arthritic joints and direct effects on cartilage to reduce cartilage breakdown (Denko, 1985; Villiger *et al.*, 1993; Walker *et al.*, 1997). Cannabinoids have been reported to have analgesic, anti-inflammatory and immunosuppressive effects and were also shown to reduce joint tissue damage in animal models of arthritis (Burstein, 2000; Malfait *et al.*, 2000). The aim of this study was to determine whether opioids and cannabinoids have direct effects on chondrocytes to reduce cartilage breakdown and are therefore chondroprotective.

The effects of opioids met- and leu-enkephalin and morphine sulphate, and cannabinoids anandamide and R-(+)-win 55,212-2 (win-55) were studied on unstimulated and IL-1 α -stimulated chondrocyte nitric oxide (NO) production and cartilage explant proteoglycan breakdown. For the cannabinoids, studies were also carried out to determine concentration ranges over which they were not toxic to chondrocytes and cartilage explants using the MTT assay to determine effects on cultured cell numbers and the lactate dehydrogenase (LDH) assay to determine cell membrane integrity in cartilage explants. Bovine articular chondrocytes were prepared by sequential enzymic digestion and plated directly into 24-well plates at high density ($2 \times 10^5/\text{cm}^2$). After 5 days they were incubated with opioids (0.1–100 μM), win-55 (0.01–100 μM) or anandamide (1–100 μM) in the presence or absence of IL-1 α (100 U/ml) for 48 h. NO was determined in culture media using the Griess reagent. Cartilage proteoglycan breakdown was determined using cultured bovine nasal cartilage explants, unstimulated or stimulated to resorb with IL-1 α (500 U/ml) in the presence or absence of opioids (1–100 μM) or cannabinoids (5–100 μM) for 2 or 4 days.

Studies of the effects of opioids indicated variable effects on IL-1-stimulated NO production in bovine articular chondrocytes: either no effect, inhibition or slight stimulation. The effects of opioids on IL-1 stimulated cartilage proteoglycan breakdown generally showed little significant effect. MTT assays showed that anandamide and win-55 at more than 10 μM were toxic to the cells; however, no toxic effects of anandamide and win-55 (10–100 μM) were detected on cartilage explants using the LDH assay. Win-55 (1 μM) significantly reduced IL-1-stimulated NO production in bovine chondrocytes and anandamide and win-55 (5–100 μM) inhibited IL-1-stimulated cartilage proteoglycan degradation in bovine cartilage explants.

Opioids had no consistent effects on the chondrocyte systems used; however, our studies indicate that cannabinoids, at concentrations which are not toxic to cells or explants, may be chondroprotective, possibly in part by inhibition of NO production.

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Effects of NSAIDs on cartilage in osteoarthritis

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Previously long-term use of some NSAIDs that are potent prostaglandin (PG) synthesis inhibitors, e.g. indomethacin, has been associated with acceleration of joint damage that includes reduction in the concentrations of cartilage proteoglycans (PrGns) (Rainsford *et al.*, 1972; Rashad *et al.*, 1989, 1992). Our initial (Phase I) studies with indomethacin and azapropazone (Rashad *et al.*, 1989, 1992) suggested that the reduced cartilage PrGns in the hip joints of osteoarthritis (OA) patients undergoing arthroplasty, on long-term treatment with 2 NSAIDs might be related to differences in inhibitory effects of these drugs on PrGn synthesis which in turn might be related to inhibition of PG synthesis (Rainsford *et al.*, 1972). In a later, as yet unpublished Phase II study, in hip OA arthroplasty patients the effects of a larger number of NSAIDs as well as analgesics and no treatments were studied using a modification of the original protocol (Rashad *et al.*, 1989). Cartilage PrGn concentrations were compared with PG concentrations. It was found that the changes in PrGn concentrations were linearly correlated with PG concentrations. This suggests that inhibition of PrGn production might be related to potency of PG inhibition by unselective COX-1/COX-2 inhibitors. *In vitro* studies that will be reviewed (including those in Rainsford *et al.*, 1997, 2003) provide further support for this concept. Increase in IL-1 and TNF α by some NSAIDs (e.g. indomethacin) in response to PG inhibition which has been shown in porcine synovial tissue in organ culture and by other authors monocyte/macrophage systems may account in part for the acceleration of cartilage destruction by NSAIDs by negative control by PGs of PrGn turnover. Some drugs (e.g. oxaprozin; Rainsford *et al.*, 2003) do not affect synovial production or actions of pro-inflammatory cytokines (IL-1,

TNF α) and some (e.g. nimesulide) reduce their production even though high rates of uptake of the drugs into cartilage is observed *in vitro*. These and other data suggest that reduction in PGs by potent inhibitors of COXs may lead to increase in pro-inflammatory cytokine production so accelerating cartilage PrGn destruction in OA.

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SESSION 6:

Novel Aspects of Chronic Diseases

32**Arthritic syndromes that defy diagnosis**

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The catalogue of rheumatic diseases comprises more than 100 diseases, most of which are well defined and therefore can be readily or ultimately be diagnosed. Some controversy exists over osteoarthritis, only a small proportion of which produces clinical symptoms of sufficient severity. The most common rheumatic manifestations, however, all of which can be grouped under the rubric, chronic pain, lack definition, as in fibromyalgia, are classified by anatomic location, as in non-specific low back pain or whiplash, are grouped by their common antecedents, as in gulf war illnesses, or focus on a single (usually unverifiable) characteristic, as in chronic fatigue syndrome. The likelihood is that these are all similar, even the same, aspects of the far end of the chronic pain distribution curve, complicated by social, cultural, and psychological features. All attempts at segregating them from the rest of chronic pain have failed, and purported abnormalities of blood constituents and other diagnostic tests are shared with many who do not manifest the symptoms, so are neither specific nor sensitive. As the symptoms are all self-reported, they have given rise to support groups and organizations whose *raison d'être* requires that these be separately diagnosed and have a poor prognosis, and in countries with an excess of lawyers give employment to those that would link the manifestations to some preceding event and supposition of progressive worsening. All the symptoms occur in similar proportions in all climes and cultures, but only in countries or areas where the reimbursement system countenances it are the diagnoses made. Thus, medicalization leads to symptom amplification and prolongation, and converts everyday events into diseases.

33**Immunopathogenesis of autoimmune diseases as a basis for therapeutic targets**

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Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease of the central nervous system (CNS) characterised by perivascular inflammatory cell infiltrates, consisting of T cells and monocytes, and plaques of demyelination as well as neuronal loss. Chemokines have been shown to play an important role in the activation and directional migration of cells to sites of inflammation. The

action of chemokines requires the expression of their complementary receptors by their target cells. In addition to this role in inflammatory cell recruitment, which is central to the pathology of a number of autoimmune diseases, chemokines also have been proposed to play a role in directing the immune response either towards to a Th1 or Th2 cell bias, resulting in a cytokine imbalance (Charles *et al.*, 1995). This has implications in autoimmunity as a number of these diseases may be the result of a cytokine imbalance. Chemokines have other functional roles including the induction of matrix metalloproteinase enzymes (De Groot and Woodroffe, 2001), which have also been demonstrated in autoimmune pathologies. The role of chemokines and their receptors in the pathogenesis of MS have been investigated using post mortem CNS tissue from MS cases as well as examining both the cerebrospinal fluid and peripheral; blood lymphocytes from patients with MS. The expression of the beta chemokine receptors CCR2, CCR3 and CCR5 in the CNS was investigated. Low levels of CCR2, CCR3 and CCR5 were expressed by microglial cells throughout control CNS tissue. In chronic active MS lesions CCR2, CCR3 and CCR5 were associated with foamy macrophages and activated microglia. CCR2 and CCR5 were also present on large numbers of infiltrating CD4⁺ T cells, indicative of Th1 cells. A smaller number of CCR3-positive lymphocytes were present, indicative of Th2 cells (Simpson *et al.*, 2000b). Previous studies have reported elevated expression of a number of chemokines in the CNS in MS lesions including CCL2, the ligand for CCR2 and CCL5 a ligand for CCR5 (De Groot and Woodroffe, 2001; Simpson *et al.*, 2000a). The elevated expression of CCR2, CCR3 and CCR5 in the CNS in MS suggests these beta chemokine receptors and their ligands play a role in the pathogenesis of MS. ELISA assays of cerebrospinal fluid samples from patients with MS showed increased CXCL10 and decreased CCL2 concentrations in the CSF. These raised chemokine levels correlated with clinical relapses in these patients. The correlation of CXCL10 levels with CD4⁺ T cell expression of CXCR3 was consistent with its chemoattractant role for activated lymphocytes (Mahad *et al.*, 2002). In a longitudinal study of 18 MS patients, an increase in CD4⁺ T cell expression of CXCR3 was noted in four patients at or near the time of relapse, the remaining MS patients remained clinically stable throughout the 7 month study and showed no significant changes in the expression of CXCR3 on the peripheral blood cell CD4⁺ T cells.

Thus, these studies suggest a key role for chemokines and their receptors in the pathogenesis of MS. As well as directing inflammatory cell recruitment, their induction of matrix metalloproteinase enzymes will further assist in cell recruitment through degradation of the extracellular matrix. Their role in biasing the Th1/Th2 phenotype in MS is yet to be explored, although the predominant phenotype in inflammatory cell infiltrates in active lesions in the CNS are of the Th1 phenotype. Further studies in MS and other autoimmune diseases may elucidate the role of specific chemokines in this proposed biased in T-cell phenotypes in autoimmunity and highlight potential therapeutic targets. From the studies to date on MS, CXCL10

neutralising agents and CXCR3 receptor antagonists may provide good therapeutic targets for treatment of MS.

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Inflammation in the pathogenesis of Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia in the elderly and is a major public health problem facing developed economies as increasing longevity contributes to increasing AD incidence. At present there is no specific preventative approved for AD and the specific treatments provide time-limited symptomatic relief at best. The major constituents of the classic neuropathological lesions of AD have been identified. Much evidence points to a crucial role for the amyloid precursor protein (APP) in the pathogenesis of AD — encapsulated in the amyloid cascade hypothesis. The key enzymes involved in APP proteolysis have been identified and their suitability as targets for AD therapeutics have been considered. Epidemiological evidence has implicated inflammation in AD pathology and treatment with anti-inflammatory drugs reduces the risk for AD. The implications of what is known about AD pathogenesis for prophylaxis and treatment of AD will be considered.

SESSION 7:

Advances in Inflammation Pharmacology

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Mechanisms of action of paracetamol and related analgesicsGARRY G. GRAHAM^{1,2}, JEREMY S.-A. KEH^{1,2} and KIERAN F. SCOTT^{1,2}¹ School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia² Department of Clinical Pharmacology, St Vincent's Hospital, Darlinghurst, NSW 2010, Australia

Paracetamol is stated frequently to be a weak inhibitor of prostaglandin (PG) synthesis. This statement is derived from studies on isolated cyclooxygenase-1 (COX-1) and COX-2 in broken cell preparations. However, paracetamol can be a potent inhibitor in intact cells. It is most potent when PGE₂ is synthesized from low concentrations of added arachidonic acid or when the substrate is released from endogenous phospholipids (Boutaud *et al.*, 2002; Graham *et al.*, 2001). The IC₅₀ values of paracetamol are often below 10 μ M, well within the therapeutic range of plasma concentrations. This potent effect of paracetamol in intact endothelial cells is overcome by increased levels of hydroperoxides indicating a possible cause of the selectivity of paracetamol (Boutaud *et al.*, 2002). At low concentrations of arachidonic acid, the pathway involving COX-2 is mostly responsible for PG synthesis when both COX-1 and COX-2 are present in cells (Graham *et al.*, 2001). It therefore follows that paracetamol may selectively inhibit PG synthesis involving COX-2, not because paracetamol is inherently more selective for the enzymes in the COX-2 pathway but, rather, because the lower flux through this pathway produces lesser levels of the hydroperoxide, PGG₂, than the pathway involving COX-1. Thus, selective inhibition of the pathway producing low levels of hydroperoxide is consistent with the apparent COX-2 selectivity of paracetamol.

There are parallels between the pharmacology of paracetamol, salicylate and the pyrazolone non-steroidal anti-inflammatory drugs, such as phenylbutazone, dipyrone, antipyrine and aminopyrine. Salicylate shows the same pattern of dependence on the levels of hydroperoxides as paracetamol and is a weak inhibitor of PG synthesis in broken cell preparations. Dipyrone is also a much more potent inhibitor of PG synthesis in intact cells containing COX-2 than cells in a cell free system (Campos *et al.*, 1999), the pattern seen with paracetamol and salicylate.

Common features of paracetamol and the pyrazolones are that they are metabolized to reactive compounds and that they inhibit PG synthesis by intact cells containing a splice variant of COX-1. The variant enzyme is termed COX-3 because the drugs inhibit the production of PGE₂ more potently in cells containing the COX-1 variant, than in cells containing COX-1. There was negligible activity on cells containing COX-2 (Chandrasekharan *et al.*, 2002). However, it is difficult at this stage to attribute the therapeutic effects of paracetamol or the pyrazolones to inhibition of COX-3 because these drugs potentially inhibit PG production by mammalian cells when COX-2 is responsible for PG synthesis.

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Recent advantages in the kallikrein–kinin system in inflammatory disorders

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Several components are involved in the formation and metabolism of the kallikrein–kinin system (KKS) in order to regulate its cellular and molecular actions. The kinins are pharmacologically very potent inflammatory polypeptides, which are released in various tissues and body fluids as results of the enzymatic actions of tissue and plasma kallikreins on kininogens (Sharma, 1991). This presentation will focus mainly on the role of KKS in joint inflammation. All the components of the KKS are present in higher concentrations in inflammatory disorders, suggesting their roles in pathophysiology of inducing hyperalgesia, arthritis, inflammatory bowel disease and asthma (Sharma *et al.*, 2000). Kinins act on B1 and B2 receptors that might cause release of other powerful non-cytokine and cytokine inflammatory mediators, e.g. prostaglandins, leukotrienes, interleukins (ILs), platelets activating factor and tumor necrosis factor from polymorphonuclear leukocytes, macrophages, endothelial cells and synovial tissue in the process of joint inflammation (Sharma and Buchanan, 1994). These inflammatory mediators are able to cause bone and cartilage damage, hypertrophic synovitis, vessels proliferation, inflammatory cell migration and probably angiogenesis in pannus formation in rheumatoid joints (Ljunggren and Lerner, 1990). In addition, B2 receptors are reported to be present in the synovial tissue, and are upregulated by IL-1 (Bathon *et al.*, 1992). Kinin antagonists have been observed to possess the potent anti-inflammatory properties. A specific B2 receptor antagonist, when infused via the ileac artery (100 nmol/h) for 2 h, results in significant inhibition of rat paw inflammation (Burch and DeHaas, 1990). We have demonstrated that a B2 receptor antagonist results in reduction of acute and chronic joint inflammation in rats (Sharma and Wirth, 1996; Sharma *et al.*, 1998). It is proposed that the development of a compound with specific antagonism of both B1 and B2 receptors might be most valuable in achieving therapeutic goals of treating joint diseases.

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Novel developments in therapy of inflammatory diseases

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The important role played by numerous cytokines, including IL-1 and TNF α , in the pathology of rheumatoid arthritis (RA) has resulted in numerous approaches to modulate these inflammatory mediators. First generation cytokine inhibitors that include monoclonal antibodies and soluble receptors have proven effective yet expensive. Several signal transducing pathways that control the activity or the production/release of these cytokines are generating considerable attention in part because the approach is amenable to small molecule development and because a number of cytokines can be inhibited simultaneously.

The induction of proinflammatory genes often results from the increased activity of NF- κ B and AP-1, two transcription factors that have become attractive targets for the development of novel anti-inflammatory drugs. NF- κ B coordinates the expression of numerous soluble proinflammatory mediators including cytokines (IL-1, TNF α , IL-6) chemokines, adhesion molecules as well as inducible enzymes (COX-2, iNOS). The NF- κ B signaling pathway has generated numerous targets that are amenable to drug discovery efforts. These include a large molecular weight complex consisting of I κ B kinases (IKK1, IKK2) and adaptor proteins (IKK γ , IKKAP1 or NEMO), and an E3 ligase selective for phosphorylated I κ B. IKK2 inhibitors including CC-839 have been shown to block the cellular production of cytokines and adhesion molecules and to be efficacious in models of arthritis (adjuvant, CIA) and are being developed clinically to treat RA, MS and cancer.

Several mitogen-activated protein kinase (MAPK) cascades are involved in inflammation and joint destruction. The MAPK referred to as Jun N-terminal kinase (JNK) activates the transcription factors c-Jun and ATF2 and other members of the Jun family that are components of the AP-1 transcription factor complex. The JNK signaling pathway is involved with cell stress response, growth, differentiation and apoptosis.

We have identified several classes of selective JNK inhibitors, including SP600125, which demonstrated significant inhibition of JNK1, -2 and -3. SP600125 is a reversible ATP-competitive inhibitor with >20-fold selectivity vs. a range of kinases and enzymes tested. In cells, SP600125 dose-dependently inhibited the phosphorylation of c-Jun, the expression of inflammatory genes COX-2, IL-2, IFN- γ and TNF- α , and prevented the activation and differentiation of primary human CD4 cell cultures.

In vivo, JNK inhibitors effectively block TNF α production induced by LPS, allergen induced asthma and adjuvant arthritis. They are also effective against experimental ischemia-reperfusion injury in the rat.

Thalidomide is a drug with known anti-inflammatory/immunomodulatory activity that is known to suppress TNF α production while enhancing IL-10 *in vitro* and *in vivo*. Since the drug has several adverse side effects including somnolence, peripheral neuropathy, constipation and congenital malformations, analogs of thalidomide (IMiDs) have been developed to circumvent these side effects. The IMiDs including CC-5013 and CC-4047 are more potent than thalidomide at suppressing TNF α production and enhancing IL-10, suppress IL-1, and have demonstrated clinical activity in a variety of cancers. Their anti-inflammatory potential is also being investigated.

Small molecule inhibitors of signal transduction, such as IKK2 inhibitors, JNK inhibitors and thalidomide analogs have the potential to be added to the armamentarium of drugs to treat RA.

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Insight into the molecular basis of selective COX-2 inhibition

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Establishing the molecular basis of selective cyclooxygenase (COX) inhibition began with the determination of the X-ray crystal structure of COX-1 in 1994, followed by the X-ray structure of COX-2 in 1996 (Garavito, 2001). Since then, mutagenesis experiments, enzyme kinetics, structure-activity-relationships and molecular modelling experiments have helped us to understand on a molecular level the substrate and inhibitor recognition by the COX isoforms, thereby providing insight into the selective inhibition of COX-2.

Interestingly, selective COX-2 inhibitors belong to at least eight different structural classes (Van Ryn *et al.*, 2000). COX-2 inhibitors of each class use distinct parts of the COX active site (COX-channel) and in the past it was difficult to understand why structurally very diverse inhibitors acted at the same enzyme with a similar mechanism. The information from X-ray analyses and from mutagenesis experiments now allow the use of molecular modelling approaches to successfully

Table 1.

Critical amino acids of COX-1 and COX-2 for the interaction with arachidonic acid and NSAIDs

Amino acid	Enzyme site	Function
Tyr 385	Catalytic site	H-abstraction at C13 of arachidonic acid
Ser 530	Aspirin site	Acetylation by aspirin
Phe 518 (Ile/Val 434)	Flexible space COX-2	Secondary shell variation: flexible channel volume in COX-2
Leu 384 (Phe/Leu 503)	Extra space COX-2	Secondary shell variation: larger channel volume in COX-2
Ile/Val 523	Side pocket COX-2	Primary shell variation allows access to the side pocket in COX-2
His/Arg 513	Side pocket COX-2	Secondary shell variation: binding in the side pocket in COX-2
Arg 120, Tyr 355	Constriction site	Ionic/H-bonding interaction with arachidonic acid and NSAIDs

gain insight into the molecular mechanism of selective COX-2 inhibition by four different structural classes of inhibitors. The advantage of molecular modelling approaches is that the recognition process of substrates and inhibitors by COX isoenzymes can be visualized and rationalized (Trummlitz and Van Ryn, 2002).

Only a small number of amino-acid substitutions are responsible for the differences in the active site of COX-2 compared to COX-1: Four structural elements responsible for selective COX-2 inhibition have been identified (Table 1).

The flexibility of Phe518 in COX-2 (caused by substitution of Val434 in COX-2 for Ile434 in COX-1) is responsible for the COX-2 selectivity of the enolcarboxamide meloxicam and the sulfanilides NS398 and nimesulide (Trummlitz and Wittneben, 2001). The space opened by Leu384 in COX-2 (caused by the substitution of Leu503 in COX-2 for Phe503 in COX-1) allows access to the extra space at the top of the COX-2 channel for selective COX-2 inhibitors of the aryl-acetic and -propionic acids. The substitution of Val523 in COX-2 for Ile523 in COX-1 influences the access to the side pocket of the active site, additionally the substitution of His513 in COX-1 by Arg513 in COX-2 influences the substrate and inhibitor binding. COX-2 inhibitors of the diarylheterocycle class (e.g. celecoxib) bind into this area (Kurumbail *et al.*, 1996). The recognition process of substrates and inhibitors by COX isoenzymes can be visualised by applying algorithms to describe local properties on the active site of the enzyme surface, thus allowing key differences in structure to be studied that may confer differential sensitivity to inhibitors. An algorithm that represents channel and cavity surfaces by colour coding local hydrophobicity (Heiden *et al.*, 1993) has been applied to visualize the interaction within the COX channel. The concept of colour-coding molecular surfaces is very helpful for the discussion of specific intermolecular interactions. In this way quantities describing the electrostatic potential, local hydrophobicity, surface topography, surface flexibility and other properties can be visualized on the surface, especially at the active site of enzymes.

Additionally, it is now possible to apply the results from structural studies of the recognition process of COX inhibitors by the COX isoenzymes to the virtual screening of large compound databases. This opens new possibilities for identifying inhibitors using the three-dimensional active site of COX-2. Of major importance for the precision of the docking experiments is the inclusion of the protein flexibility into the calculations. First attempts to incorporate the protein flexibility instead of the rigid crystal structures have greatly improved the results of such *in silico* testing. It was possible to match virtual screening results with experimentally determined activities of COX-2 inhibitors from the diarylheterocycle series. The rank order of activities from *in silico* binding to COX-2 showed good correlation to experimentally determined activities of these inhibitors. The application of these techniques to *in silico* screening of large databases opens new possibilities for identifying inhibitors using the three-dimensional active site of COX-2. As these techniques only became available after the discovery of the currently available selective COX-2 inhibitors, it will be interesting to follow up the strategies involved in discovery of future COX-2 inhibitors. In the pursuit of new highly selective COX-2 inhibitors one should be aware that an open issue remains: Is there an optimal degree of COX-2 selectivity for clinical application?

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COX-2-selective agents: the bad and not so bad news

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The development of COX-2-selective agents promised equal efficacy to conventional NSAIDs with vastly improved gastrointestinal safety. Enthusiasts claim that both objectives have been achieved while others take a more guarded stance. Here we report on the gastrointestinal consequences of selective COX-2 absence and in-

hibition in the mouse and the effect of NSAIDs and COX-2-selective inhibitors on patients with inflammatory bowel disease (IBD).

The finding that COX-1 deficiency and selective inhibition of COX-1 with SC-560 in mice is not associated with gastrointestinal damage seriously challenges the dogma that COX-1 alone plays an important pathophysiological role in NSAID-induced gastrointestinal damage. During an intensive breeding program to obtain homozygous COX-2 knockouts it was observed that about 15% of these animals died suddenly from intraperitoneal sepsis. A systematic study of these animals showed that half had evidence of increased small bowel permeability (51CrEDTA) with intestinal inflammation (faecal granulocyte marker protein). Those with the most severe inflammation had microscopic ileocaecitis that is distinctively different from NSAID enteropathy. The same pathology was seen in animals receiving celecoxib for over 3 months. These findings suggest that it is COX-2, and not COX-1, that is important for maintenance of intestinal integrity. Patients with IBD are purported to experience clinical relapse of disease when taking NSAIDs. The incidence and mechanism of this action is unknown. We administered Naproxen (classical NSAID), nabumetone (a non-acidic pro-NSAID that does not have a topical effect), nimesulide (COX-2-selective agent without a topical effect) and paracetamol as a control to 15–30 patients with IBD. Both naproxen and nabumetone caused clinical relapse of disease in 20% of patients with a corresponding increase in intestinal inflammatory activity within a week of administration. Nimesulide and paracetamol caused no clinical relapse at this time. The results suggest that the relapse of IBD is not mediated by selective inhibition of COX-2 or the topical effect, but may be mediated by selective COX-1 inhibition or dual inhibition of both COX-1 and -2.

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Cyclooxygenase-2 function is essential for bone fracture healing

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Bone fracture healing occurs by tissue regeneration. The same osteogenic pathways used to form the skeleton during fetal development are employed to make new bone at the fracture site and thereby heal the broken bone. Endochondral ossification is the process in which a cartilaginous precursor tissue is replaced with bone. This process is used to make the long bones of the skeleton. Endochondral ossification is also the principal osteogenic pathway used during fracture healing. Mesenchymal cells that migrate and proliferate at the fracture site form a cartilaginous tissue that is rapidly replaced with bone. In addition, direct bone formation, or intramembraneous ossification, occurs at the periphery of the fracture site to form a buttress that helps to mechanically stabilize the soft cartilaginous tissue until it has been replaced

with bone. Though the bone formation pathways used during fracture healing and fetal development are strikingly similar, the molecular and physiological events that initiate and maintain osteogenesis in each circumstance remain largely unknown.

Hypoxia and inflammation are the initial physiological events following a bone fracture that do not occur during fetal osteogenesis. Blood flow is disrupted leading to local hypoxia and hematoma formation. Inflammation soon follows and occurs prior to mesenchymal cell accumulation at the fracture site (callus). We hypothesized that the inflammatory reaction initiates the fracture healing cascade by release of growth factors and other biological active compounds at the fracture site. In support of our hypothesis, previous studies had shown that non-steroidal anti-inflammatory drugs (NSAIDs) could delay healing of bone fractures in experimental animals (Rø *et al.*, 1976; Allen *et al.*, 1980). However, it was unclear from these studies whether the delay in healing was caused by inhibition of COX-1, COX-2, or a reduced inflammatory reaction.

As an initial test of our hypothesis, retired-breeder, male, Sprague–Dawley rats were treated with COX-2 selective NSAIDs, celecoxib (4 mg/kg) and rofecoxib (3 mg/kg), prior to and continuing after a closed femur fracture (Simon *et al.*, 2002). Fracture healing was assessed by radiography, histology, and torsional mechanical testing and was compared to rats treated with indomethacin (1 mg/kg) or control (no drug) rats. We found that fracture healing proceeded normally in the control rats and was delayed in the indomethacin-treated rats as expected (Rø *et al.*, 1976; Allen *et al.*, 1980). In contrast, none of the rats treated with celecoxib or rofecoxib completely healed their fractures even after 8 weeks. X-ray scoring showed a significant decrease in healing between control and rofecoxib-treated rats (0.75 ± 0.05 S.E.M. vs. 0.38 ± 0.04 S.E.M., respectively). Torsional mechanical testing showed significant decreases in all parameters (peak torque, rigidity, shear stress, and shear modulus) between the control and rofecoxib-treated rats. However, no significant differences in the mechanical properties of the fractured femurs from celecoxib-treated rats were found despite the fact that all of the celecoxib-treated rat femurs failed as non-unions or incomplete unions. Our histological analysis demonstrated that NSAID treatment disrupted the normal morphology of the cartilage elements present in the early fracture callus (2 weeks post-fracture). In the COX-2 inhibitor treated rats, chondrocytes were absent in the 3-week post-fracture calluses indicating that endochondral ossification and therefore normal fracture healing had ceased.

The data showed a clear inhibitory effect of the COX-2 inhibitors on fracture healing in rats. The simplest explanation was that loss of COX-2 activity impaired healing. However, to eliminate the possibility that the COX-2 inhibitors themselves were inhibiting fracture healing by some non-specific mechanism, fracture healing was assessed in COX-1 knock-out and COX-2 knock-out mice. Fracture healing was found to proceed normally in the COX-1 knock-out mice but failed in the COX-2 knock-out mice. The COX-2 knock-out mice formed a cartilaginous fracture callus with little or no histological evidence of new bone formation.

Ongoing experiments are aimed at defining when inhibition of COX-2 is most detrimental to fracture healing. For these experiments, female, Sprague–Dawley rats were used. The half-life of celecoxib in female rats is approximately 12 h which is similar to that for humans and much greater than the approximately 4 h half-life of celecoxib and rofecoxib in male rats (Paulson *et al.*, 2000). The rats were treated with 4 mg/kg of celecoxib once daily for 5 days prior to fracture, or for 5, 10, 15, 21 or 28 days post-fracture. We found no difference in fracture healing between control rats and the rat treated with celecoxib for 5 days prior to fracture. In contrast, radiographic assessment and mechanical testing at 8 week after fracture, showed that the healing success decreased with duration of COX-2 inhibition. Histology showed that COX-2 inhibition led to pre-mature chondrocyte differentiation in the fracture callus which stops endochondral ossification. We interpret this data to indicate that COX-2 functions at two points in the fracture healing process. First, inhibition of COX-2 during the inflammatory phase (days 1–7 post-fracture) reduces fracture healing success but is not as severe as prolonged inhibition of COX-2. Second, COX-2 is necessary during the endochondral phase of fracture healing (days 10–21 post-fracture) and appears affect chondrocyte differentiation.

We also made a startling observation as part of ongoing experiments. Rats were anesthetized with a standard IP injection of ketamine (40 mg/kg) and xylazine (5 mg/kg). We observed that approx. 5% of the rats from all the treatment groups, except the pre-treatment group, died from the anesthesia (7 of 137). In contrast, approximately 31% of the rats pre-treated with celecoxib for 5 days with the last dose of celecoxib administered 4 h prior to anesthesia died (7 of 22 rats). We currently have no explanation for this observation but believe it is noteworthy.

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Age-related decrease in gastroprotection

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Introduction

The age-related increase in the incidence of gastric and duodenal ulcer disease has been well documented (Kurata *et al.*, 1985). There are numerous reports on the

increased susceptibility of gastric mucosa in aged animals (Gronbach and Lacy; 1995; Miyake *et al.*, 1996).

The increased susceptibility of rat gastric mucosa was explained by alteration of gastric defence mechanism: it was shown that the release of endogenous prostaglandins (PG), nitric oxide (NO) and/or calcitonin gene-related peptide (CGRP) decreased in aged animals. These three substances were supposed to be involved in modulation of gastric mucosal integrity, probably through interaction with each other (Whittle *et al.*, 1990).

Previously we showed, that alpha-2 adrenoceptor agonists (Gyires *et al.*, 2000) as well as opioid receptor stimulants exerted gastroprotective effect given either peripherally (subcutaneously) (Gyires *et al.*, 1997) or centrally (intracerebroventricularly or intra-cisternally) (Gyires *et al.*, 2000; Gyires and Rónai, 2001) to the rat and both NO and PGs were supposed to be involved in the gastroprotective effect of these substances (Gyires *et al.*, 2000; Gyires and Rónai, 2001). The age of the rats used in these experiments was 6–10 weeks.

The question was raised how the gastroprotective effect of opioid peptides may be altered in aged (21–25 months old) rats. In the first step, however, we aimed to investigate the gastroprotective action induced by opioids in young mature rats of different ages (6–8 and 14–16 weeks), namely the age of young rats was very different in the light of literature: 2–6-month-old rats have been used as a young animals. Therefore, first it had to be clarified whether there is difference in gastric mucosal protective processes in young mature rats of different ages.

Methods

Male Wistar rats weighing 160–420 g (6 weeks to 16 weeks old) were used.

Ulcer model: After 24 h starvation the mucosal damage was induced by 0.5 ml acidified ethanol given orally. One h later the stomachs were removed and examined for lesions.

Analgesia assay: The analgesic effect was measured by using the tail-flick test originally described by D'Amour and Smith (1941). The test measures the time required to respond to heat stimulus.

The compounds were injected either orally (p.o.), subcutaneously (s.c.), intracerebro-ventricularly (i.c.v.), or intracisternally (i.c.).

The plasma ACTH and corticosterone levels were determined by radioimmunoassay from truncal blood of the rat.

Results

1. The acidified ethanol-induced gastric mucosal lesions were much more severe in 14–16-week-old rats (ulcer indexes 60 vs. 98).
2. The mucosal protective effect of DADLE (0.8 nmol/rat), deltorphin II (3.3 nmol/rat), DAGO (0.2 nmol/rat) and β -endorphin (0.01 nmol/rat) observed in 6–8-week-old rats was abolished in 14–16-week-old animals.

3. Similarly, the gastroprotective effect of DADLE, DAGO and β -endorphin following i.c. administration observed in 6–8-week-old rats was highly reduced in 14–16-week-old animals.
4. DAGO and DADLE reduced the mucosal lesions in 6–8-week-old rats when given s.c. However, the protective effect of the maximally effective doses of DADLE (825 nmol/kg s.c.) and DAGO (960 nmol/kg s.c.) was decreased in 14–16-week-old rats by about 50%.
5. Both PGE₂ (280 and 560 nmol/kg) and capsaicin (1600 and 3200 nmol/kg) inhibited the gastric mucosal lesions in 6–8-week-old rats when given orally. In 14–16-week-old animals the gastroprotective effect of capsaicin was abolished, the action of PGE₂ was less influenced.
6. DADLE induced an analgesic effect in the tail-flick test when given i.c.v., the ED₅₀ value was 3.5 nmol/rat in 6–8-week-old rats. In contrast, no analgesic effect was produced by the dose of 40 nmol/rat i.c.v. in 14–16-week-old animals.
7. ACTH (59.9 ± 4.3 pmol/ml) and the corticosterone (598 ± 11 pmol/ml) plasma levels were significantly higher in 14–16-week-old rats than that in 6–8-week-old rats (25.5 ± 2.3 and 277 ± 13 pmol/ml, respectively).

Conclusions

The gastroprotective effect of different opioid peptides given centrally (i.c.v. or i.c.) dramatically decreased, and also reduced mucosal protective action was observed following peripheral (s.c.) administration in 14–16-week-old rats. The analgesic action of DADLE injected i.c.v. was also diminished. The results suggest that both central and peripheral mechanisms may be involved in the reduced gastroprotective effect observed in 14–16-week-old rats.

Acknowledgements

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Direct evidence for the exclusion of desensitization to capsaicin during two weeks treatment in human healthy subjects

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Backgrounds: Indomethacin (IND) produces gastric mucosal damage in human healthy subjects, producing an increase of gastric microbleeding (Moron *et al.*, 2003). It was also proved that the application of capsaicin is able to prevent IND-induced gastric mucosal bleeding (Mózsik *et al.*, 2003). IND acts *via* the inhibition of COX-1 and COX-2 (0.30; Johns, 1999). Many researches suggest the existence of the desensibilisation of polymodal afferent nerves to capsaicin during the lifespan, producing different gastrointestinal disorders.

Aims: (i) To study the effectivity of capsaicin on the IND-induced gastric mucosal damage in human healthy subjects before and after a chronic (2 weeks) capsaicin treatment; (ii) to compare the extents of IND-induced gastric microbleedings without and with administration of capsaicin (200 and 400 μ g) intragastrically before chronic administration of capsaicin (3×400 μ g, i.g.) and (iii) to prove or to exclude the presence of desensitization of polymodal afferent nerves to capsaicin in human healthy subjects during the chronic, two weeks (3×400 μ g, i.g.) capsaicin treatment.

Materials and methods: The observations were carried out in 14 human healthy subjects (aged 40 ± 10 years). They had no complaints, and had negative physical status and well as laboratory parameters. The extent of IND (3×25 mg/day)-induced gastric mucosal microbleedings (without and with application of capsaicin in doses of 200 and 400 μ g, i.g.) was measured by the measurement of gastric microbleeding using the method of Hunt *et al.* (1979). The results were repeated on the same human healthy subjects after the two weeks treatment with capsaicin (3×400 μ g i.g.), when the extent of IND-induced gastric mucosal bleeding (without and with application of 200 and 400 μ g given orally) was calculated. The extent of the gastrointestinal bleeding was expressed in ml/day (as means \pm S.E.M.; $n = 14$). The observations were carried out according to good clinical practice (GCP). The human healthy subjects were randomized to the entry of the observations, however all human subjects received the same schematic treatment and went over the all observations. Ethical Permission was obtained from the Regional Ethical Committee of Pécs University, Pécs, Hungary (April, 1997).

Results: (i) IND produced the same extent of gastric microbleeding before and after the 2 weeks of capsaicin treatment (without acute administration of capsaicin 400 μ g i.g. ED₅₀ = 400 μ g of capsaicin on the inhibition of gastric acid secretion);

(ii) the acutely applied capsaicin (given intragastrically in different doses) dose-dependently prevented the IND-induced gastric microbleedings; (iii) the extent of the IND-induced gastric microbleedings and the dose-dependent preventive effects of capsaicin were the same before and after 2 weeks of capsaicin ($3 \times 400 \mu\text{g/day}$ orally) treatment.

Conclusions: Because the extent of IND-induced gastric microbleedings and the capsaicin preventive effect (in a dose-dependent manner) was found the same extent before and after the 2 weeks of capsaicin treatment, we exclude the development of desensibilisation of polymodal afferent nerves to capsaicin. The statement of the 'decreased sensibilisation of capsaicin-sensitive afferent nerves' has to be reconsidered in the evaluation of the polymodal capsaicin-sensitive role for evaluation of their possible ethiological role in the development of different gastrointestinal disorders.

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Adverse reactions from NSAIDs and analgesics: approaches for prevention

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Gastrointestinal (GI) ulceration, bleeding and symptomatic reactions, hepatic, renal and skin reactions constitute the main adverse reactions (ADRs) associated with both the established NSAIDs, as well as new coxibs. While there have been some improvements in GI safety with the coxibs, there are still some risks of upper GI bleeding/ulceration and the symptoms (epigastric disorders, etc.) are still of frequent occurrence. For elderly subjects at risk of GI ADR's there is still need for ulcer protective agents. Some patients self-administer with a range of natural products (nutraceuticals) either as alternative therapies to obviate ADR's from NSAIDs or as a means of overcoming the lack of therapeutic benefit of existing drugs. Some of these natural products (e.g. celery seed extract) have the advantage of being ulcer protective/preventative as well as having anti-inflammatory analgesic activities (Whitehouse *et al.*, 2001).

A major problem with some but not all NSAIDs is their potential for causing ulceration and related complications (peritonitis, diaphragm disease) in the lower intestinal tract. Studying these reactions in humans is difficult because of the inevitable invasive nature of procedures and the relative paucity of knowledge of basic biochemical and cellular processes. Models in rodents have proven useful but these species do not have intestinal functions that more closely resemble those in humans. Our recent studies (Rainsford *et al.*, 2003) have shown the value of using pigs to study intestinal ulcer disease, e.g. induced by indomethacin. These studies have shown that there is a disconnect between GI bleeding and ulcer development. This may relate to the differences among NSAIDs with their propensity to delay or inhibit platelet-regulated thrombotic reactions. These and fundamental aspects of the mechanisms of actions of NSAIDs enable selection of drugs for being of high or low risk of bleeding, risks of lower c.f. upper ulcer choice of appropriate preventative and protective procedures for application 'at risk' subjects.

Hepatic reactions are of considerable concern as is their widespread occurrence with most NSAIDs. The mechanisms of their development are multifactorial and there are frequent complications from patient-related factors (e.g. prior or concurrent diseases, concurrent hepatotoxic drugs). NSAID-related mechanisms are drug-specific but include drug- or metabolite-covalent modifications of endogenous proteins and other biomolecules, induction of mitochondrial changes leading to apoptosis, altered pharmacokinetics and a range of biochemical changes involving effects of intermediary, fatty acid and protein/nucleic acid metabolism. The hepato-renal syndrome which occasionally occurs can be related to complex reactions involving impairment of renal function and consequent reduced clearance of NSAIDs and dose-related impairment of hepatic metabolism of the drugs.

Cutaneous reactions from NSAIDs are probably the least-understood of the common ADRs. Associations with the development of reactive metabolites has been implicated from a number of studies and accumulation of these and/or parent drug in the skin leading to activation of resident macrophage histocytic populations may form part of the initiation of immune-based cutaneous reactions.

Prevention of serious ADRs from NSAIDs can often be achieved by (1) reducing exposure to other drugs or their use in certain confounding disease states that are likely cofactors in the aetiology of ADRs, (2) optimisation of dosage in relation to pharmacokinetic properties of the drugs, (3) exploiting potential benefits from protective natural products and (4) focus on mono-drug therapy or where combinations are essential for therapy those combinations where rigorous data are available to support their use.

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SESSION 8:

Poster Presentations

P1**Comparison of dexibuprofen and ibuprofen in post-operative dental pain: a controlled clinical trial**

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The pain resulting from the surgical removal of one or more impacted third molars is a validated pain model. The pain in this model is both predictable and consistent, beginning one to three hours after surgery and ranging in intensity from moderate to severe (Cooper, 1983). Nonsteroidal anti-inflammatory drugs (NSAIDs), e.g. ibuprofen and S(+)-ibuprofen (dexibuprofen, the purified active enantiomer of ibuprofen) are effective in the relief of this pain (Dionne and McCullagh, 1998) and their use is widespread. There is clinical evidence that S(+)-ibuprofen provides faster onset and greater analgesia than racemic ibuprofen, but without decreased duration of activity or greater incidence of adverse reactions (Dionne and McCullagh, 1998). In this parallel group, double-blind, double-dummy, randomised, active- and placebo-controlled clinical trial, we compared the efficacy and tolerability of single doses of dexibuprofen (400 mg and 200 mg) in a special crystal form versus ibuprofen (400 mg) and placebo in patients suffering from moderate to severe pain due to the unilateral extraction of an impacted third molar in the lower jaw.

In total 344 patients (42% male, 58% female) were enrolled into the clinical trial and the 4 treatment groups were comparable with respect to age, gender, weight and height. The primary efficacy criterion was sum of pain intensity difference (SPID), secondary criteria were TOTPAR, peak PID, time to peak PID, total number of hours over which baseline pain was reduced by at least 50%, global assessment of efficacy and time/number of used escape medication. Single doses of ibuprofen 400 mg and of both doses of dexibuprofen (400 mg and 200 mg) showed superiority versus placebo. Furthermore, equivalent efficacy of 200 mg dexibuprofen and of 400 mg ibuprofen was proven. A positive dose-response relationship between 200 mg and 400 mg dexibuprofen was shown. Dexibuprofen 400 mg yields the highest magnitude of pain reduction and has the fastest onset of pain relief. The secondary criteria were in accordance with the main criterion. Hence, 84% (400 mg dexibuprofen), 72% (200 mg dexibuprofen), 79% (400 mg ibuprofen) and 24% (placebo) of patients rated the global subjective assessment of efficacy as very good or good. Only one adverse event occurred during the observation period (placebo group).

These results show that a dose ratio of 0.5:1 of dexibuprofen vs. ibuprofen provided adequate analgesia for this very intense post-operative pain, despite the partial unilateral inversion from the inactive R(–)-ibuprofen into the active moiety (S-enantiomer). Furthermore, a dose–response relationship for dexibuprofen was shown. The results suggest that dexibuprofen in a dose of 400 mg, as well as of 200 mg, in this special crystal form is a safe and effective treatment for post-operative dental pain.

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P2

Pro-inflammatory cytokines induce anabolism *via* induction of BMP-2 in mouse knee joints

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Proinflammatory cytokines IL-1 β and TNF- α are known for their catabolic activities on articular chondrocytes. However, we have recently shown that, in human osteoarthritis (OA) cartilage, pro-inflammatory cytokines can also increase biosynthetic activities through induction of BMP-2 (Fukui *et al.*, 2003). Interestingly, previous reports indicate that when IL-1 β is injected into knee joints of mice, chondrocytes show enhanced anabolism after an initial suppression (Van Beuningen *et al.*, 1991; Van de Loo *et al.*, 1994). Taken together, these results indicate that chondrocytes also have anabolic response to pre-inflammatory cytokines as a repair mechanism. In this study, we hypothesize that in mice, as in humans, the proinflammatory cytokines induce anabolic response in articular cartilage through the up-regulation of BMP-2.

To determine the effects of cytokines on cartilage anabolism, IL-1 β and TNF- α were injected into a right knee joint of male C57B110 mice, 60–80 days old. The mice were sacrificed at 0 (control), 1, 3, 5, 9 and 14 days after injection, and synthetic activity in patellar cartilage was evaluated by incorporation of [³⁵S]sulfate. Immediately after injection, IL-1 β strongly suppressed synthetic activity in patellar cartilage, causing about 50% decrease in [³⁵S]sulfate incorporation. After initial suppression, synthetic activity recovered rather quickly, and it overshot the control level at day 5: the activity was 1.7 times above the control level. TNF- α did not cause an obvious suppression of proteoglycan synthesis in mouse knee joints: even

at the early time points after injection, the ratio of [^{35}S]sulfate incorporation was 1.3-times above the control level, and the up-regulation continued up to 9 days.

As proof of the concept that pro-inflammatory cytokines stimulate biosynthesis via induction of a BMP, the effect of noggin ($K_d = 10^{-11}$), a potent antagonist for BMPs, was analyzed in treated and untreated mouse knees. For this, IL-1 β or TNF- α solution was injected into bilateral knee joints in each mouse, and 10 μl of noggin solution containing 1 μg of recombinant noggin was injected 2 or 4 days later. Noggin suppressed proteoglycan synthesis significantly in IL-1 β treated mice at day 5. A similar trend was observed in TNF- α -treated mice, although the suppression was not statistically significant. In mice receiving IL-1 β injection, strong immunostaining of BMP-2 was observed from day 1 to day 9 in the femoral cartilage contacting the patella. In conclusion, we have shown that pro-inflammatory cytokines stimulate anabolism in human articular cartilage via induction of active BMP-2 (Fukui *et al.*, 2003). The same effect can now be seen *in vivo* by injection of IL-1 β and TNF- α into mouse knee joints. Consequently, we suggest that the pro-inflammatory cytokines may induce both catabolic and anabolic processes necessary for remodeling of cartilage leading to repair.

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P3

Dexibuprofen in the treatment of high-altitude headache: analgetic effect in the Himalayas?

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Approx. 20% to 90% of those who are not adopted to altitudes between 2500 and 5000 m will experience high-altitude headache (HAH) (Burtscher, 1999). It is well known that HAH is the predominant symptom of acute mountain sickness (AMS) frequently accompanied by nausea, vomiting and insomnia. Over 95% of AMS sufferers have HAH, usually frontal, developing within 6–8 h of arrival at altitude and lasting for up to 5 days (Broome *et al.*, 1994; Burtscher *et al.*, 1995). There is evidence that dual cyclooxygenase inhibitors like ibuprofen or naproxen are effective in treatment and prevention of HAH (Broome *et al.*, 1994; Burtscher *et al.*, 1995). A post-marketing surveillance trial in 43 patients suffering from HAH was conducted to evaluate the efficacy and tolerability of dexibuprofen (the single

effective and pure enantiomer of ibuprofen) in a special crystal form under realistic circumstances (mean baseline altitude 4386 m).

Prior to enrollment, all patients (86% male, 14% female) suffered from severe (86%) or extreme (14%) headache according to the classification of the Lake-Louise AMS score. Patients received up to 2 tablets of 400 mg dexibuprofen during the 24-h observation period. The primary efficacy criterion was pain relieve, assessed by a 100-mm visual analog scale. Intensity of headache improved from 65.7 mm at baseline to 5 mm after 12 h and to 2 mm after 24 h ($P < 0.001$). The mean subjective time to total pain relieve was 48 min. After 12 h 93% of the patients had a pain improvement of 90–100% (healing), and therefore no patient had to descend to lower altitudes. Hence, 98% of physicians and 93% of patients rated the global subjective assessment of efficacy as excellent or very good. Tolerability was rated as excellent or very good by 98% of physicians and patients. Only 1 adverse event (vomiting, sickness) occurred during the observation period.

A self-limiting effect can be almost completely excluded, since HAH is lasting up to 5 days and it is suggested to be related to hypoxia. A progression of the disease to the high altitude cerebral edema is possible. The efficacy of dexibuprofen could be explained if a prostaglandin-mediated increase in cerebral microvascular permeability is part of the pathophysiology of AMS. The results suggest that dexibuprofen in this special crystal form is a safe and effective treatment for high altitude headache (HAH).

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P4

Thiocyanate from smoking amplifies inflammatory responses in rats

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Aim: Establish a molecular basis for imputing smoking as a factor promoting arthritis (Albano *et al.*, 2001) smokers excreting more urinary thiocyanate than non-smokers (Butt *et al.*, 1974).

Methods: Rats were given tap water or water containing 10 mM sodium thiocyanate (SCN^-) a metabolite of hydrocyanic acid from tobacco smoke. Arthritis was induced in Wistar and Dark Agouti rats with (i) exogenous arthritogens = mycobacterial cell walls (M) or avridine (A) plus an incomplete adjuvant (IA) or (ii) two endogenous arthritogens = collagen (C) type-II (plus IA) or squalene, inoculated into tailbase. Chronic/fibrotic inflammation was initiated by injecting zymosan (0.3 mg) or hydroxyapatite (5 mg) in rear paws.

Results:

1. Thiocyanate increased severity of arthritis and reduced time of onset of M, A or C-induced arthritides initiated with a mineral oil (MO) adjuvant.
2. Replacing MO with oils of low adjuvanticity, e.g. fish oils, 1-octadecene, isopropyl myristate abolished the arthrogenicity of M, A and C in rats on tap water: however, rats with SCN^- supplementation developed striking arthritis.
3. Wistar rats given i.d. squalene alone developed arthritis with SCN^- supplementation.
4. Local irritant responses to intrapedal zymosan or hydroxyapatite were significantly enhanced by SCN^- supplementation.

Conclusion: Thiocyanate is another environmental factor predisposing to arthritis. Its pro-inflammatory effect may be mediated (a) in the thyroid = antimetabolite to iodide, (b) in inflammatory loci after oxidation to cyanide by myeloperoxidase (from PMNs), (c) by antagonising anti-inflammatory zinc or copper; collectively retarding normal postinflammatory healing.

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P5**AZD3582 reduces pain, inflammation and fever in rats**

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Background: AZD3582 [4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2 naphthyl) propanoate] is a COX-inhibiting nitric oxide donator (CINOD), the first in a new class of agents designed for the treatment of acute and chronic pain. AZD3582 has an innovative mechanism of action of balanced inhibition of COX-1 and COX-2 and controlled nitric oxide donation. This may provide anti-inflammatory and analgesic effects by inhibition of both COX- and caspase-regulated cytokine production, while at the same time providing the potential for improved gastrointestinal safety.

Methods: AZD3582 or naproxen at equimolar doses (0.3–100 $\mu\text{mol/kg}$), or vehicle ($n = 7-8$ per group) was administered orally 1 h before injection of carrageenan into the ankle joint of male Sprague–Dawley rats. Animals were videotaped from below and pain behaviour scored on a 4-point scale (0 = equal weight on hind paws and 3 = paw completely elevated). Anti-inflammatory efficacy was assessed by administering AZD3582 or naproxen (doses as above; $n = 5-6$ per group), or vehicle ($n = 12$), to male Wistar rats 30 min before carrageenan injection into their hind-paw footpad. Paw volume was measured immediately before and at 1-h intervals over a 5-h period following carrageenan injection. As NSAIDs only

Table 1.

Analgesic effect vs. vehicle and potencies of AZD3582 and naproxen

	AZD3582		Naproxen	
	3 h post-injection	5 h post-injection	3 h post-injection	5 h post-injection
Lowest dose with analgesic effect ($\mu\text{mol/kg}$)*	3	3	3	10
Estimated ED ₅₀ ($\mu\text{mol/kg}$), 95% CI in parentheses	1.2 (0.8–2.8)	2.9 (1.1–3.9)	1.0 (0.9–1.6)	9.2 (6.4–9.8)

* $P < 0.05$ versus vehicle.

partially reduce oedema in this model, potencies were described by ED₃₀. Fever was induced in Sprague–Dawley rats by subcutaneous injection of Brewer's yeast. Rectal temperatures were measured before and 2 h after administration of AZD3582 or naproxen (0.3, 1, 3, 10 and 30 $\mu\text{mol/kg}$) or vehicle ($n = 8$ per group).

Results: AZD3582 and naproxen dose-dependently reduced pain behaviour compared with vehicle. At 5 h post-injection, both the AZD3582 dose that had a statistically significant analgesic effect compared with vehicle, and the ED₅₀ for AZD3582, were lower than those of naproxen (Table 1).

AZD3582 and naproxen dose-dependently reduced paw oedema. Compared with vehicle, both agents had a statistically significant anti-inflammatory effect at doses of 10 $\mu\text{mol/kg}$ or higher. The ED₃₀ values of AZD3582 ($\mu\text{mol/kg}$ (95% CI)) and naproxen were 16 (8.5–37) and 16 (8–52), respectively. Injection of Brewer's yeast increased mean rectal temperature from $37.6 \pm 0.04^\circ\text{C}$ to $39.6 \pm 0.03^\circ\text{C}$ ($n = 96$). Fever was dose-dependently reduced by AZD3582 and naproxen. The lowest dose that significantly reduced fever compared with vehicle was similar: 3 $\mu\text{mol/kg}$ for AZD3582 and 1 $\mu\text{mol/kg}$ for naproxen.

Conclusions: AZD3582 produces a dose-dependent reduction in pain, inflammation and fever in rats. The clinical efficacy of AZD3582 and the ability of its multi-pathway mechanism of action to protect the gastrointestinal tract are being investigated in man.

P6

AZD3582, a COX-inhibiting nitric oxide donator (CINOD), is gastroprotective in the rat

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Background: AZD3582 [4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2 naphthyl) propanoate] is a COX-inhibiting nitric oxide donator (CINOD), developed for the treat-

ment of acute and chronic pain. NSAIDs that inhibit both COX-1 and -2 reduce the level of prostaglandins involved in pain, inflammation and haemostasis. However, inhibition of both COX isoforms can have adverse effects on the gastrointestinal (GI) mucosa (Wallace *et al.*, 2000). AZD3582 has a novel, multi-pathway mechanism of action of controlled nitric oxide donation and balanced COX inhibition. Nitric oxide has shown protective effects in the GI tract (Muscara and Wallace, 1999). Therefore, AZD3582 has the potential to provide analgesic and anti-inflammatory efficacy, while at the same time protecting the GI tract.

Methods: A series of studies assessed the GI safety profile of AZD3582 in rats. Single doses of AZD3582 and naproxen (0–100 $\mu\text{mol/kg}$) were administered orally to male Wistar rats. Segments of the GI tract were examined for macroscopically visible haemorrhagic lesions 5.5 h after dosing. In histopathological studies, AZD3582 or naproxen were administered for 2.5 days at a dose of 30 $\mu\text{mol/kg}$ b.i.d. or for 4.5 days at 30–120 $\mu\text{mol/kg}$ b.i.d. The rats were killed 1 h after the last dose. GI tract segments were fixed and examined to determine the presence/degree of ascites or adhesions, and the number of perforations. In a further study, rats were given AZD3582 or naproxen (doses increasing from 80 to 125 to 190 $\mu\text{mol/kg}$ b.i.d.) or vehicle for 18 days. Each GI tract was examined and given a score relating to the total length of all macroscopically visible haemorrhagic erosions.

Results: At equimolar doses of the two agents, the acute gastric erosion score was lower for AZD3582 than for naproxen. The UD_{30} (the dose in $\mu\text{mol/kg}$ causing a 30% incidence of gastric ulcers) was lower for naproxen than for AZD3582; 6.5 times more AZD3582 was required to induce gastric ulcers in 30% of rats. In the histopathological studies, there was a clear trend towards more irritative and corrosive effects with naproxen on the gastric and intestinal mucosa (villous oedema and increased or irregular mucin production/goblet cell occurrence) compared with vehicle or AZD3582. This was particularly noticeable in the stomach and the proximal part of the intestine. The effects of naproxen were observed at doses of 60 $\mu\text{mol/kg}$ or more, but were only seen at the highest AZD3582 dose (120 $\mu\text{mol/kg}$). In the 18-day study, no ulcers were observed in the AZD3582 and vehicle groups, while naproxen caused intestinal damage affecting 3.7% of the mucosal area examined.

Conclusions: AZD3582 has low ulcerogenic potential in rats, and a favourable GI safety profile relative to naproxen. The novel multi-pathway action of AZD3582, involving balanced COX inhibition and controlled nitric oxide donation, confers both tissue-protective effects in the GI tract and anti-inflammatory and analgesic efficacy.

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P7**Cox-1 inhibition is not essential for the development of NSAID-induced enteropathy**

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Background and aims: Inhibition of COX-1 is believed to be of pivotal importance in NSAID-induced enteropathy, but there is increasing evidence that COX-2 inhibition and the topical effect may have a synergistic detrimental action.

In order to dissociate and recombine these effects we treated rats with a selective COX-1 inhibitor (SC-560), a selective COX-2 inhibitor (Celecoxib), an uncoupler of oxidative phosphorylation that does not inhibit COX (R-flurbiprofen) and a classical NSAID with all 3 properties (indomethacin). We also compared the morphology of normal, COX-1 and COX-2-deficient mice after dosing with R-flurbiprofen (uncoupler, no effect on COX), S-flurbiprofen (uncoupler, inhibits both COX isoforms) and R-2-phenylpropionic acid (uncoupler, but not an NSAID and no COX inhibition).

Methods: Intestinal permeability (^{51}Cr -labelled EDTA), inflammation (faecal granulocyte marker protein) and macro- and microscopic appearances were assessed at baseline and after the dosing of drugs.

Results: COX-1 or COX-2 inhibition and the topical effect (using R-flurbiprofen and R-2-phenylpropionic acid at doses that do not affect intestinal prostaglandin levels) alone did not cause any significant damage to the GI tract of rats. Dual inhibition of COX-1 and COX-2 and in separate studies the simultaneous inhibition of COX-2 and the topical effect resulted in intestinal inflammation similar to that caused by indomethacin.

R-Flurbiprofen led to minimal small bowel damage in normal and COX-1 knockout mice while S-flurbiprofen consistently damaged the gut. However, administration of R-flurbiprofen to COX-2 knockout mice resulted in identical damage as S-flurbiprofen to normal or COX-2 knockout mice. R-2-Phenylpropionic acid caused GI damage in COX-2 knockout mice, but not in COX-1 knockout or normal mice.

Conclusions: Short-term selective inhibition of COX-1 or COX-2 or the topical effect does not damage the small intestine. The combined inhibition of COX-1 + COX-2 or COX-2 inhibition+the topical effect results in injury of the intestine. The latter combination shows that NSAID enteropathy can occur without COX-1 inhibition.

P8**The intestinal tolerability of AZD3582 in rats**

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Background: The COX-inhibiting nitric oxide donator (CINOD), AZD3582, has been shown to be at least as effective as an NSAID in rat models of pain and inflammation. NSAIDs that inhibit COX-1 and COX-2 maximally reduce the production of prostaglandins involved in pain, inflammation and homeostasis. However, inhibition of both COX isoforms can adversely affect the gastrointestinal (GI) mucosa (Wallace *et al.*, 2000). As nitric oxide has protective effects in the GI tract (Muscara and Wallace, 1999), AZD3582 has the potential to spare the GI mucosa through its inherent property of nitric oxide donation, while being an effective anti-inflammatory and analgesic agent.

Aim and methods: To compare the effects of AZD3582 [4-(nitroxy)butyl-(2S)-2-(6-methoxy-2-naphthyl) propanoate] with those of naproxen on small bowel ulcer counts, intestinal inflammation (assessed by changes in granulocyte marker protein) and small bowel permeability (assessed by urinary ^{51}Cr -EDTA measurement) in the rat. Male Sprague–Dawley rats ($n = 8$ per group for each assessment) were given single doses of AZD3582 or naproxen (10, 30, 100, 300 $\mu\text{mol/kg}$) or vehicle. Small bowel ulcer counts were made at 48 h. Intestinal inflammation was assessed daily from 4 days prior to and 4 days after administration of the agents. ^{51}Cr -EDTA was given 1 h after the agents and urinary levels measured at 5 h.

Results: Small bowel ulcer counts were significantly lower with AZD3582 (3.9 ± 1.5) than with naproxen (17.1 ± 2.7 ; $P < 0.001$). Significant increases in granulocyte marker protein from vehicle levels (5.6 ± 1.6 mg/l) were evident only with the highest doses of AZD3582 (59.5 ± 24.9 mg/l) and naproxen (50.9 ± 12.9 mg/l; $P < 0.001$); there was no significant difference between the agents. The 5-h urinary excretion of ^{51}Cr -EDTA increased significantly ($P < 0.001$) from vehicle ($4.2 \pm 0.5\%$) following all doses of AZD3582 ($6.3 \pm 0.7\%$, $20.9 \pm 3.0\%$, $24.0 \pm 4.1\%$, $23.2 \pm 4.2\%$) and naproxen ($9.5 \pm 1.2\%$, $19.3 \pm 1.9\%$, $28.9 \pm 1.9\%$, $24.6 \pm 4.1\%$), with no significant difference between the two drugs.

Conclusions: AZD3582 protects against small bowel ulcers, while having similar effects to naproxen on intestinal ^{51}Cr -EDTA permeability. Small bowel inflammation in rats was only seen at the highest dose of both drugs. It is likely that the low ulcerogenic potential of AZD3582 in rats is due to nitric oxide donation, which is part of its multi-pathway mechanism of action.

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P9**Intestinal hypoxia in COX-1 KO mice: down-regulation of energy metabolism enzymes**

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Background and Aims: The pathogenesis of NSAID-induced gastrointestinal damage is controversial, but most are of the opinion that COX-1 inhibition is of paramount importance. However, it is now clear that COX-1-deficient mice (or animals treated with a selective COX-1 inhibitor) do not develop intestinal ulcers spontaneously. Proponents of the COX-1 hypothesis have suggested that the lack of damage may be due to up- or down regulation of intestinal proteins that counter the effect of prostaglandin deficiency. Our aim was to compare protein expression in the gut between COX-1 knockout mice and normal mice to gain further insight into NSAID pathology.

Methods: Expression of proteins was compared between COX-1 (–/–) and COX-1 (+/+) mice with proteomics, differently expressed proteins were identified with mass spectrometry.

Results: Gel electrophoresis disclosed about 1000 proteins. Three spots representing 3 enzymes that are essential for energy metabolism were consistently found in significantly reduced amounts in COX-1 (–/–) animals, namely isocitrate dehydrogenase (3.8x), hydroxyacyl CoA dehydrogenase (3.1x) and creatine kinase (4.5x) (down-regulated). We did not detect up regulation of proteins involved in nitric oxide metabolism in the COX-1 (–/–) mice.

Conclusion: The reduced expression of key enzymes of oxidative metabolism is consistent with chronic vascular underperfusion of the intestine, which is not in itself sufficient to cause intestinal ulceration. These findings are compatible with the idea that COX-1-mediated prostaglandin production exerts its main effect on the intestinal microvasculature and hence a possible mechanism for the gastroprotective effects of synthetic prostaglandins.

P10**Biochemical mechanisms of the gastric mucosal prevention of vitamin A, β -carotene in 4 h pylorus-ligated plus sodium-salicylate-treated rats**GY. MÓZSIK¹, M. FIGLER¹, I. JURICKSKAY¹, B. GASZTONYI¹, GY. RUMI¹, Z. MATUS² and A. PÁR¹¹ First Department of Medicine, Medical Faculty, University of Pécs, Pécs, Hungary² Department of Medical Chemistry, Medical Faculty, University of Pécs, Pécs, Hungary

Backgrounds: Sodium salicylate is a well-known chemical component, which damages the gastric mucosal damage in presence of free gastric H^+ secretion (Davenport *et al.*, 1970). After that time we proved the existence of a positive correlation between the suggested gastric H^+ backdiffusion and the decreased gastric mucosal energy metabolism (Mózsik *et al.*, 1981). We also proved the gastric mucosal preventive effects of vitamin A and beta-carotene are independent processes from the inhibitory effects their gastric acid secretion. The aims of this study were: (i) To study the gastric mucosal preventive effects of vitamin A and β -carotene in the 4 h pylorus-ligated plus sodium salicylate (200 mg/kg, i.g.)-induced gastric mucosal damage; (ii) to evaluate the changes in the gastric mucosal level of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenylate pool (ATP + ADP + AMP), 'energy charge' $((ATP + 0.5 ADP)/(ATP + ADP + AMP))$ and cyclic adenosine monophosphate (cAMP) in 4 h pylorus-ligated plus sodium-salicylate-treated animals in association of the development of their gastric mucosal preventive effects against sodium salicylate.

Materials and methods: The observations were carried out of Sprague–Dawley rats of both sexes, weighing 180–210 g. The animals were treated with 20 mg/kg sodium salicylate in 4 h pylorus-ligated rats without and with i.g. administration of vitamin A and β -carotene (0.01, 0.1, 1.0 and 10 mg/kg). The animals were killed 4 h after the start of the examinations. The number and severity were calculated. The severity of gastric mucosal damage was calculated using a semiquantitative scale (Mózsik *et al.*, 1982). The tissue concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were enzymatically measured (Boehringer-Ingelheim, Germany), while the tissue content of the gastric mucosal level of cAMP was measured by radioimmunoassay (RIA, Beckton Dickinson, Orangeburg, USA). The protein content was measured by biuret reaction. The adenylate pool (ATP + ADP + AMP) and 'energy charge' $((ATP + 0.5 ADP)/(ATP + ADP + AMP))$ were calculated according to Atkinson (1968). The results are expressed as means \pm SEM in accordance to 1 mg protein ($n = 10-20$).

Results: The gastric secretory volume, gastric acid and gastric acid output was the same in pylorus-ligated and pylorus-ligated plus saline (2 ml, i.g.) treated animals. No ulceration was detected, and no difference was obtained between the biochemical parameters (gastric mucosal level of ATP, ADP, AMP, adenylate pool, 'energy charge' and cAMP). Also, the number and severity of gastric mucosal damage and volume of the gastric secretory volume increased significantly, meanwhile the gastric acid output, gastric mucosal levels of ATP, ADP, AMP, adenylate pool, 'energy charge' and cAMP decreased significantly in 4 h pylorus-ligated plus aspirin-treated rats; meanwhile, the value of the 'energy charge' remained unchanged. The gastric mucosal preventive effects against the sodium salicylate decreased dose-dependently. The number and severity of gastric mucosal damage, volume of gastric secretory responses (without modification of gastric

acid output), on the other hand, dose-dependently increased the gastric mucosal levels of ATP, ADP, AMP, adenylyate pool and cAMP in the pylorus ligated plus sodium salicylate treated rats, meanwhile the values of 'energy charges' remained unchanged.

Conclusions: The sodium-salicylate-induced gastric mucosal damage associated with the significantly decreased adenosine phosphate metabolism in 4 h pylorus-ligated rats. Vitamin A and β -carotene dose-dependently prevent the gastric mucosal damage in association with the significant increase of adenosine phosphate metabolism. The gastric mucosal preventive effects of vitamin A and β -carotene differ from the decrease of the gastric acid secretion. The sodium-salicylate-induced gastric mucosal damage and the preventive effects of vitamin A and β carotene depend only the gastric mucosal energy metabolism. Vitamin A and β carotene dose-dependently decreased the volume of gastric secretory responses without any modification of gastric acid. This fact suggests that the carotenoids are able to modify only the volume of gastric volume (water) secretory responses. Calculation of the 'energy charge' should be considered as the research pool for use to indicate the tissue metabolism.

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