

INFLAMMOPHARMACOLOGY

9th International Conference September 8–10th 2008 Queens' College, Cambridge UK

This Programme and Abstracts is from the ninth in the series of conferences on INFLAMMOPHARMACOLOGY (Side Effects of Anti-inflammatory Drugs). Details of the previous conferences in this series can be found in the Appendix.

The conference was held at Queens' College, Cambridge on 8th to 10th September, 2008.

As in the past, our goal was to have presentations and discussions among widely specialists from academe, industry and medical practice to review the current status of chronic and acute inflammatory diseases and their therapy. There was particular focus on some key issues concerning the safety and efficacy of NSAIDs; the clinical, physiopathological and molecular aspects of inflammation and pain; and therapeutic interventions exploiting the links between inflammation and cancer as well as neurodegenerative conditions such as Alzheimer's disease (e.g. from use of various NSAIDs having differing modes of action).

We are most grateful to the generosity of Sponsors. In these difficult financial times we appreciate the financial assistance and donations from these organizations as well as from registrations of those who came to the conference at their own expense. The sponsors (in alphabetical order) were:

Bioronica Ltd, the Department of Pharmacology, University of Cambridge, M/P Biomedical Consultants LLC, Heffers Ltd, Helsinn Healthcare SA, Pfizer UK Ltd and Queens' College Cambridge.

None of the sponsors had any influence upon the choice of speakers and poster presenters or the content of the conference.

Organising Committee

Brian A Callingham (Organising Secretary, University of Cambridge & Queens' College, Cambridge, UK), Richard H Hunt (McMaster University, Hamilton, ON, Canada), Walter F Kean (McMaster University, Hamilton, ON, Canada), Michael C Powanda (M/P Biomedical Consultants LLC, Mill Valley, CA, USA), Michael P Seed (William Harvey Research Institute, Queen Mary, University of London, London, UK) and Kim D Rainsford (Sheffield Hallam University, Sheffield, UK).

PROGRAMME

Monday 8th September

"Festschrift" in honour of Professor Kim Rainsford, commemorating his contributions to the field of inflammopharmacology

The speakers on this day were those past and current collaborators or associates of Kim Rainsford.

Opening remarks and welcome from the co-chairmen:
Drs Brian Callingham and Michael Powanda

Prof. Richard Hunt
McMaster University, Hamilton, Ontario, Canada
"Gastrointestinal and cardiovascular consequences of COX inhibition: What is the role of immunomodulation?"

Prof. Ingvar Bjarnason
Guy's, King's and St Thomas' Hospital, London, UK
"Non-COX factors in the gastrointestinal damage caused by NSAIDs"

Prof. Nicholas Moore
Department of Pharmacology, Université Victor Segalen
Bordeaux 2, Bordeaux cedex, France
"Clinical toxicology trials and the development of NSAIDs"

Prof. Walter Kean,
McMaster University, Hamilton, Ontario, Canada
"Gold complexes"

Opening remarks from the co-chairmen: Profs. Walter Kean and Ian Rodger

Prof. Michael Whitehouse
University of Queensland and Griffiths University, Brisbane,
Queensland, Australia
"Revisiting the Conditional Concept for pathology, pharmacology and toxicology"

Professor Garry Graham
University of New South Wales & St Vincent's Hospital
Darlinghurst, New South Wales, Australia
"Relationships between effects and plasma concentrations
of non-steroidal anti-inflammatory drugs and paracetamol"

Prof. David Parkinson
Biomedical Research Centre, Sheffield Hallam University,
Sheffield, U.K.
"Inflammation in the pathogenesis of Alzheimer's Disease"

Prof. Richard Chegwidzen
LECOM, Erie, Pennsylvania, USA
"The carbonic anhydrases: potential therapeutic targets in
renal cell carcinoma"

Dr Brian Callingham
Department of Pharmacology, University of Cambridge,
Cambridge, U.K.
"Some actions of nitric oxide-donating NSAIDs on isolated
preparations of vascular and other smooth muscles"

Dr John Gillard
Aegara Inc., Nun's Island, Montreal, Que., Canada
"A Tribute to Kim, my PharmacoDaemon"

Dr Michael Powanda
M/P Biomedical Consultants LLP, Mill Valley, CA, USA
"Reflections on a career"

After dinner, Dr Lisa Wagner from the Victoria and Albert
Museum, London gave an illustrated talk on, "Vigani, the
first Cambridge Professor of Chemistry (1702 in the Julian
Calendar) and his cabinet in Queens' College".

Tuesday 9th September

"Pain and Inflammation"

Opening remarks from the co-chairmen: Profs Tony Milton
and Garry Graham

Prof. Peter McNaughton
Department of Pharmacology, University of Cambridge,
Cambridge, U.K.
"Why pain gets worse - molecular mechanisms of inflamma-
tory heat pain"

Prof. Ian Rodger
St Joseph's Hospital and McMaster University, Hamilton,
Ontario, Canada
"Analgesic Targets: Today and Tomorrow"

Prof. Rod Flower
William Harvey Research Institute, Queen Mary, University
of London
"Anti-inflammatory drugs and the annexin A1-ALX receptor
system"

Prof. Alan McComas
McMaster University, Hamilton, Ontario, Canada
"The Janus faces of migraine"

"Arthritic Diseases"

Opening remarks from the co-chairmen: Dr Brian Hazleman
and Prof. Walter Kean

The Watson Buchanan Memorial Lecture

Professor George Nuki
University of Edinburgh
"Treating osteoarthritis in 2008: evidence, expert opinion
and commonsense"

Dr Frances Hall
Addenbrooke's Hospital and University of Cambridge
"Modifying cardiovascular risk in rheumatoid arthritis"

Prof. Ann Parke
University of Connecticut Health Center at St Francis Hospi-
tal and Medical Center, Hartford CT, USA
"The many talents of hydroxychloroquine"

Prof. Peter Lees
Royal Veterinary College, University of London
"PK/PD integration and PK/PD modelling of NSAIDs for
dosage determination in the cat"

"Inflammation and GI injury"

Opening remarks from the co-chairmen: Profs. Richard Hunt
and Stefan Laufer

Prof. Tom Brzozowski
Jagiellonian University Medical College, Cracow, Poland
"Gastric adaptation to aspirin: physiological mechanisms
and clinical impact"

Prof. Ludmila Filaretova
Laboratory of Experimental Endocrinology, Pavlov In-
stitute of Physiology, Nab. Makarova, 6, St. Petersburg,
199034, Russia
"Dual action of glucocorticoid hormones on the gastric mu-
cosa: how the gastroprotective action can be transformed to
an ulcerogenic one"

Prof. Klara Gyires
Semmelweis University of Medicine, Budapest, Hungary
"Pharmacological analysis of α_2 -adrenoceptor subtypes medi-
ating analgesic, anti-inflammatory and gastroprotective actions"

Prof. Kim Rainsford
Sheffield Hallam University, Sheffield
"Protection against NSAID-Related Adverse Reactions by
Multiple Stratagems"

Free communications

Dr Rajan Radhakrishnan
College of Pharmacy, University of Southern Nevada, South Jordan, Utah and Department of Anesthesiology, University of Utah, USA.

“Development and characterization of an animal model of chronic pelvic pain due to prostate inflammation”

Dr Len Lichtenberger
Department of Integrative Biology & Pharmacology,
The University of Texas Health Science Center at Houston,
Houston, Texas, USA

“Effect of NSAIDs on the hydrophobic phospholipid barrier of the GI tract and the development of the PC-NSAID technology”

Debate**“Safety, efficacy and evolving uses of OTC analgesics”**

Chairmen: Profs Laurie Prescott and Kim Rainsford

Initiating speaker: Prof. Randall Harris
College of Medicine and Public health, The Ohio State University, Columbus, Ohio USA

“Selective and non-selective cyclooxygenase-2 (COX-2) inhibitors in cancer chemoprevention and therapy: safety and efficacy”

Topics included when to use ibuprofen or other NSAIDs in preventing cancers and Alzheimer’s and vascular dementias.

Special Pre-dinner Lecture: Prof. Walter and Mrs Mary Kean

McMaster University Faculty of Health Sciences, Hamilton, Ontario, Canada

“Forensic Anthropology, ‘Oetzi’ - The Simulian Iceman (ca. 5,300 yr BP)”

Following dinner, an informal presentation (with samples on hand) was given by Professor Nicholas Moore (Department of Pharmacology, Université Victor Segalen Bordeaux 2, Bordeaux cedex, France) entitled, “How nice stuff can be good medicine: the pharmacological and therapeutic properties of Armagnac”.

Wednesday 10th September**“Chronic Inflammatory Diseases and Cancer”**

Opening remarks from the co-chairmen: Profs. Ingvar Bjarnason and George Nuki

Prof. Michael Langman
University of Birmingham, Birmingham.

“Cyclooxygenase inhibition and cancer treatment. A chance missed?”

Profs Maije Eglite and Kim Rainsford (with Dr Andrejs Skesters) Riga Stradins University & Institute of Environmental and Occupational Health, Paul Stradins Hospital, Riga, Latvia & Sheffield Hallam University, Sheffield, U.K.
“Clinical aspects of health disturbance tendencies of the Chernobyl Nuclear Power Plant Clean-up Workers from Latvia”

Dr Michael Seed
Queen Mary, University of London
“Intracellular signalling pathways controlling inflammation: potential for pharmacological control of chronic diseases”

Dr Derek Gilroy
MRC Sepsis Cooperative, University College London, Rayne Institute, London
“Resolution of inflammation: prospects for controlling chronic diseases”

Prof. Nicholas Bellamy
Mayne Medical School, The University of Queensland, Brisbane, Australia
“Electronic data capture using cellular technology: implications for clinical trials and practice”

Closing remarks – Dr Brian Callingham & Dr Michael Powanda

List of poster presentations**Clinical Investigations****P 1**

Women with pain due to osteoarthritis: efficacy and safety of a once-daily formulation of tramadol

W. F. Kean, S. Bouchard and R. Gossen

P 2

Safety and efficacy of intramuscular sodium ribonucleinate [Osteochondrin® S] for relief of pain and joint functions in knee osteoarthritis

G. Stommel, W. W. Bolten, K. D. Rainsford, K.-H. Schühlein, A. Dempsey, J. Schnitker

P 3

Autoimmune diseases revisited

A. L. Russell

P 4

Effects of ibuprofen and anti-oxidants on biochemical and clinical aspects of health status of chernobyl clean-up workers from Latvia

T. Zvagule, A. Skesters, M. Eglite, J. Reste, N. Kurjane, N. Gabruseva, K. D. Rainsford

P 5

Effects of antioxidant mixtures on biochemical (oxidant/antioxidant) status and clinical observations in chernobyl clean-up workers from latvia

J. Reste, T. Zvagule, A. Skesters, M. Eglite, N. Kurjane, N. Gabruseva, K. D. Rainsford

Experimental Therapeutics**P 6**

Gastrointestinal (GI) and cardiovascular (CV) risks associated with the use of anti-inflammatory drugs are influenced by TH1/TH2 dichotomy: a novel concept

I. T. Padol, R. H. Hunt

P 7

How safe is paracetamol as an alternative analgesic to NSAIDs?

M. W. Whitehouse, K. D. Rainsford, P. Ghosh

P 8

Intestinal permeability and inflammation in rats given indomethacin and antibiotics

C. M. Hotz-Behofsits, R. Simpson, I. Bjarnason

P 9

Methylamine increases nitric oxide synthase activity of rat white adipocytes: a new role for the semicarbazide-sensitive amine oxidase?

B. Romagnani, L. Fracassini, C. Alfarano, R. Pirasino, L. Raimondi

P 10

Mechanisms of the analgesic effect of corticotropin-releasing factor on the somatic pain sensitivity in rats

N. I. Yarushkina, T. R. Bagaeva, L. P. Filaretova

P 11

Inhibition of receptor activator NF- κ B (rankl) induced osteoclast formation by histone deacetylase inhibitors

M. D. Cantley, P. M. Bartold, V. Marino, D. P. Fairlie, D. R. Haynes

P 12

Cannabinoid receptor activation induces apoptosis in colon cancer cells through $\text{tnf}\alpha$ and ceramide

F. Cianchi, L. Magnelli, M.C. Vinci, C. Lanzi, E. Ronconi, E. Masini

P 13

Human whole blood assay for rapid and routine testing of non-steroidal anti-inflammatory drugs (NSAIDs) on cyclooxygenase-2 activity.

S. Luik, C. Greim, F. Dehner, S. S. Ayoub, S. Laufer

P 14

Effects of nimesulide on drg neurons: inhibition of PKC- ϵ translocation and of substance p synthesis and release

V. Vellani, P. Sacerdote, S. Franchi, M. Prandini, G. Pavesi, M. Bianchi

P 15

Anti-rheumatic activity and cytokine synthesis inhibition by the oligosulphated disaccharide sucrose octasulphate.

M. Lees, L. Dugo, Z. Ganiyu-dada, J. Mancini, M. Burnet, M. Seed

P 16

Some *in vitro* actions of nitric oxide releasing non-steroidal anti-inflammatory agents on cervine and ovine digital artery rings

O. L. I. Davies, I. S. Gregory, C. Rhodes, R. Turner, A. S. Milton, B. A. Callingham, K. D. Rainsford

P 17

Ribonucleate sodium [Osteochondrin S[®]] inhibits cytokine-induced cartilage-bone degradation but not proteoglycan synthesis

K. D. Rainsford, A. Jonas, C. Ying, F. C. Smith

Novel Therapeutic Agents**P 18**

A novel compound from celery seed with a bactericidal effect against *helicobacter pylori*

Y. Zhou, B. Taylor, T. J. Smith, M. Clench, N. Davies, K. D. Rainsford

P 19

Therapeutic colloidal silver: time for re-appraisal?

M. W. Whitehouse, C. L. Brown, D. E. Butters, G. L. Hancock, G. R. Bushell

Topical Abstracts**P 20**

Is the pathogenesis of 'binge drinking' an inflammatory-mediated process?

R. J. Ward, D. T. Dexter, P. de Witte, F. Lallemand, F. Scholl, C. Eberhardt, M. A. Colivicchi, C. Ballini, L. Della Corte

P 21

p38 MAP kinase triggers dendritic or accessory cells in the allogeneic mixed lymphocyte and Con-A – induced lymphocyte responses.

V. Moradi, E. Johnson, L. Dugo, V. Holan, M. Burnet, S. Laufer, M. Seed

P 22

A simple score for assessing bone erosion in rodent arthritic paws visualised by micro focal computer tomography X-ray.

J. Mancini, M. R. Jones, C. Alam, M Burnet, M. P. Seed.

Appendix

Inflammopharmacology Conference Series (with Side-Effects of Anti-inflammatory Drugs Symposia) Conference Details and Publications of Proceedings

1. Side-Effects of Anti-inflammatory / Analgesic Drugs
(K. D. Rainsford and G. P. Velo, Organisers)
Venue: University of Verona (Italy), September 1982
Publication, Book: Side-Effects of Anti-inflammatory / Analgesic Drugs. *Advances in Inflammation Research*, 6. Editors: K. D. Rainsford and G. P. Velo. Raven Press, New York, 1984
2. Side-Effects of Anti-inflammatory Drugs
(K. D. Rainsford and G. P. Velo, Organisers)
Venue: University of Cambridge & Queens' College, Cambridge (UK) 31st July to 2nd August, 1985
Publication, Book: Side-Effects of Anti-inflammatory Drugs, 2 Part Volumes
Editors: K. D. Rainsford and G. P. Velo. MTP Press, Lancaster, 1985.
3. Side-Effects of Anti-inflammatory Drugs
(K. D. Rainsford and G. P. Velo, Organisers)
Venue: University of Verona (Italy), 8th – 11th May, 1991
Publication, Book: Side-Effects of Anti-inflammatory Drugs, 3.
Editors: K. D. Rainsford and G. P. Velo. Kluwer Academic Publishers, Lancaster, 1992
4. Side-Effects of Anti-inflammatory Drugs
(K. D. Rainsford, Organiser)
Venue: Sheffield Hallam University, 7–9th August, 1995
Publication, Book: Side-Effects of Anti-inflammatory Drugs, IV

Editors: K. D. Rainsford and G. P. Velo. Kluwer Academic Publishers, Dordrecht, 1997

Publication (peer-review papers): *Inflammopharmacology*, Vol 3, pp 137–204 & 311–399, 1996.

5. Side-Effects of Anti-inflammatory Drugs
(K. D. Rainsford & M. C. Powanda, Organisers)
Venue: South San Francisco Conference Center, 17th–19th March, 1997
Publication, Book: Safety and Efficacy of Non-Prescription (OTC) Analgesics and NSAIDs,
Editors: K. D. Rainsford & M. C. Powanda, Kluwer Academic Publishers, Dordrecht, 1998.
6. International Conference on Inflammopharmacology & (K. D. Rainsford & M. C. Powanda, Organisers)
6th Symposium on Side Effects of anti-inflammatory Drugs.
Venue: Chateau Elan, Braselton. Georgia, USA, 23rd–26th May, 1999.
Publication: *Inflammopharmacology*, Vol 7, pp 191–303, 339–350.
7. International Conference on Inflammopharmacology & (K. D. Rainsford & M. C. Powanda, Organisers)
7th Symposium on Side Effects of anti-inflammatory Drugs.
Venue: Sheffield Hallam University, Sheffield, 10th–13th September, 2001
Publication: *Inflammopharmacology*, Vol. 9, pp. 101–216.
8. International Conference on Inflammopharmacology & (K. D. Rainsford & M. C. Powanda, Organisers)
8th Symposium on Side Effects of anti-inflammatory Drugs.
Venue: Royal College of Physicians of Edinburgh,
Edinburgh, 22nd–24th April, 2003.
Publication: *Inflammopharmacology*. Vol 11, pp. 301–470.

S1

Gastrointestinal (GI) and cardiovascular (CV) consequences of COX inhibition: what is the role of immunomodulation?

Richard H. Hunt and Ireneusz T. Padol

Department of Medicine, McMaster University, Hamilton, Ontario, Canada.

GI intolerance is experienced by ~50 % of those taking NSAIDs and may lead to life threatening events including ulcer and bleeding. NSAID-induced GI consequences are directly correlated with gastric prostaglandin inhibition (Rainsford *et al.* 1995), particularly PGE₂. They are acute and physiological in nature and their onset can be measured in hours or days. NSAID-related GI complications are positively influenced by PPIs, *H. pylori* eradication and by use of DMARDs. In contrast, concomitant use of SSRIs has a detrimental effect with an increased risk of GI bleeding in patients concomitantly taking NSAIDs or aspirin. The introduction of coxibs resulted in an approximate halving of GI risk however, evidence of increased CV risk resulted in the withdrawal of several of these drugs from the market. Soon after the withdrawal of rofecoxib several outcome studies indicated that a similar CV risk is seen with traditional non-selective NSAIDs (tNSAIDs).

Several hypotheses have been presented to explain the mechanisms for the increased rate of MI with anti-inflammatory drugs with the prothrombotic effect given most attention. However, the prothrombotic hypothesis has to be seriously questioned: there is no evidence of increased deep venous-renal-portal- or hepatic vein thrombosis and only one study suggesting an increase in thrombotic stroke. Rather, the observation is of MI with most of these non-fatal and MI is associated with unstable atherosclerosis. The adverse effect of MI appears to evolve over a long time period as most occur after 12 weeks or more of anti-inflammatory drugs (Kearney PM *et al.*, 2006), rather than acutely, as would be expected if thrombosis was the cause. Moreover, the increase in the incidence of MI is seen with many tNSAIDs, which inhibit prostaglandin production by COX-1 and COX-2 (Warner and Mitchell, 2008).

Prostaglandins play a role in immunomodulation by skewing the immune response away from Th1. Atherosclerosis is a Th1 driven immune disease and unstable atheromatous plaques are characterized by the presence of typical Th1 type immunocytes such as: T-cells, neutrophils and macrophages. Thus, we hypothesize that prostaglandin inhibition with both COX-2 selective and tNSAIDs results in augmentation of the Th1 response (Padol and Hunt, 2008). This immunomodulation by anti-inflammatory drugs evolves slowly, exacerbating any Th1 driven atherosclerosis, which leads to plaque instability, and consequently to MI. Aspirin, with its predominant COX-1 inhibition seems to overcome CV risk and offers some form of protection in CV diseases, particularly thrombosis. However, this protection comes with the cost of profoundly increasing GI bleeding, even at the low-doses (McQuaid and Laine, 2006).

The current impasse in our understanding of the predominant mechanism(s) of the CV consequences of COX inhibition calls for basic and clinical studies to better elucidate the broad biological consequences and time dependence of prostaglandin metabolism and COX inhibition.

References

- Rainsford, K. D. et al (1993) *Agents & Actions* **39**, C21–C23.
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 McQuaid, K. R. and Laine, L. (2006), *Am J Med* **119**, 624–38.

S2

Non-COX factors in the gastrointestinal damage caused by NSAIDs.

Ingvar Bjarnason

King's College Hospital, London, UK

With the discovery of cyclooxygenase (COX) it was postulated that the therapeutic actions and gastrointestinal complications of NSAIDs could both be explained by their inhibition of the COX enzyme. However, there were a number of experimental inconsistencies such as why rectally administered aspirin did not cause gastric damage in animals, despite causing over 90 % reduction in mucosal prostaglandin levels. This led to the concept of a topical damaging action of NSAIDs. The pathogenesis was thought to be due to either a NSAID-surface membrane phospholipid interaction or “trapping” of acidic NSAIDs within the gastric cells and enterocytes, but the consequence of the latter remained uncertain. In an attempt to elucidate the non-COX factors in the development of mucosal damage sub-cellular organelle marker studies were carried out in rat small bowel after indomethacin. Results showed that the brush border and mitochondria were selectively affected (Somasundaram *et al.*, 1997). Furthermore, electron microscopy studies showed mitochondrial changes pathognomic for either inhibition of electron transport or uncoupling of mitochondrial oxidative phosphorylation. Further studies in coupled rat liver mitochondria showed that all acidic NSAIDs uncouple mitochondrial oxidative phosphorylation (OxP). The potency correlated inversely ($r = -0.87$) with the pKa of the NSAID (Mahmud *et al.*, 1996). Non-acidic NSAIDs, including esterified NSAIDs, and COX-2 selective agents (alkaline pKa) did not uncouple OxP. A number of studies showed that in the small bowel NSAID-induced uncoupling reduced intracellular ATP levels, which led to increased intestinal permeability. Increased intestinal permeability is the central mechanism by which the biochemical – cellular damage is translated to tissue damage. This was verified in man, by showing that non-acidic NSAIDs, such as nabumetone, did not increase

small intestinal permeability while all conventional NSAIDs did (Bjarnason *et al.*, 1993). Furthermore, based on the proposed mitochondrial action of NSAIDs, indomethacin was administered to volunteers with increasing amounts of glucose and citrate, the substrates for glycolysis and the Krebs cycle, respectively (Bjarnason *et al.*, 1992). This combination significantly reduced, in a dose-dependent fashion, the intestinal permeability increase due to indomethacin providing further evidence for the damaging uncoupling action of NSAIDs. This combination has also been shown to reduce gastrointestinal blood loss in humans given indomethacin in the short-term. Lastly, a review of short-term endoscopy studies in man showed a significant correlation between the pKa of NSAIDs and gastric damage while the damage did not relate significantly with measures of mucosal prostaglandins or in vitro COX-1/COX-2 selectivity (Bjarnason *et al.*, 2007).

With the emergence of the COX-2 selective agents the potential importance of the “topical” effect in the damage was again ignored. Nevertheless selective COX-1 inhibition or absence has no significant detrimental effect on the gastrointestinal tract while long-term COX-2 absence or inhibition is associated with severe small bowel, but not gastric, damage. This damage is increased by concomitant COX-1 inhibition or the topical effect. Indeed NSAID-induced gastrointestinal damage can be brought about without a concomitant reduction in mucosal prostaglandins.

References

- Bjarnason, I. *et al.* (1993) *Gastroenterology* **104**, 1832–47
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 Mahmud, T. *et al.* (1996) *Arthritis Rheum* **39**, 1998–2003
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S3

Clinical toxicology trials and the development of NSAIDs

Nicholas Moore

Dept of Pharmacology, University of Bordeaux, Bordeaux, France

NSAIDs are among the most commonly used drugs, and among the most studied. Their effects are perfectly described, both positive (analgesia, antipyrexia, anti-inflammatory) and negative (upper and lower GI, renal, possibly cardiovascular toxicity), and are the object of several thousand papers. They are symptomatic drugs whose main indications are osteoarthicular diseases, both inflammatory (RA) and degenerative (OA), and common pain of any source. Their activity is consistent across drugs, most differences being related to doses used, to plasma half-life, and to COX1/COX2 selectivity, since though COX2 inhibition mostly covers the anti-inflam-

matory effects, analgesia is also COX1 dependent. The main concern with these drugs, until recently, was in fact GI risk, related to COX1 inhibition. GI toxicity will depend upon intensity of COX1 inhibition, drug half-life, and the concomitant use or not of gastroprotective agents.

Recommendations on the development of NSAIDs for OA, RA and other chronic diseases, devised by expert committees not including the GPs that prescribe the vast majority of NSAIDs, call for clinical trials lasting a sufficient time, the recommended duration being 6 months to 1 year in the European Medicines Evaluation Agency's CPMP/EWP/784/97 recommendation dating from 1998, which also refers to 3CC17a dating from 1987. A new document (July 2008) however recommends the revision of these documents.

These initial recommendations resulted in typical 3- to 6-month clinical trials of full-dose NSAIDs in OA that showed a high rate of adverse GI reactions, about 1%. From these results, quantitative modelling estimated that chronic NSAIDs use results in about about 4000 deaths per year in the UK, 18000 in the US (Tramer *et al.*, 2000). Unsurprisingly, results from epidemiological studies that showed much lower rates in real life use were discarded as being unreliable evidence. These suggested high numbers of possible deaths resulted in the development of the (relatively) specific COX2 inhibitors, whose safety was confirmed in two large-scale clinical trials against full-time full-dose NSAIDs for a year, with event rates of about 1% in the NSAIDs arms, 0.5% in the coxib groups (Bombardier *et al.*, 2000; Silverstein *et al.*, 2000). This justified the marketing and widespread use of Coxibs. Rofecoxib was later removed from the market when it appeared that the higher dose of 50 mg/day used for analgesia in the US market was associated with a higher rate of myocardial infarction than comparators (Graham *et al.*, 2005). Since then it has been showed that the risk of myocardial infarction was increased with all NSAIDs to about the same degree as with 25 mg/day rofecoxib, i.e. a relative risk around 1.3.

In real-life studies, the event rates for GI bleeding is about one tenth to one fortieth that expected from clinical trials (Moore, 2001): why? In fact, NSAIDs, even in OA which represents about a third of users (RA is about 3%, the rest is common acute painful conditions) are used intermittently, for about 100 days per year, and/or rarely at the doses tested in the clinical trials (Moore, 2003). In addition, gastroprotection in the form of proton pump inhibitors (PPI) is used in 20 to 60% of NSAIDs users (including COX2 inhibitors), especially when there are identified risk factors (age, previous GI events, long-term use of NSAIDs) (Depont *et al.*, 2007). NSAIDs with PPI do not seem to carry a greater risk of GI bleeding than COX2-selective inhibitors, or than the background population (Chan *et al.*, 2002). In fact the whole COX2 development was driven by identification of a real but vastly overestimated risk because of very unnatural clinical trial conditions, akin to clinical toxicology studies: were these studies designed to go beyond demonstrating proof of concept or the unsafe nature of NSAIDs, the methods would include p.r.n. usage of NSAIDs to obtain symptom relief, with concomitant use of PPI in higher risk patients. The results and conclusions of the studies might have been very different, and we might have avoided the Coxib debacle.

References

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S4

Gold sodium thiomalate treatment for rheumatoid arthritis

W. F. Kean¹ and K. D. Rainsford²

¹ Division of Rheumatology, McMaster University, Hamilton, Ontario, and

² Biomedical Research Centre, Sheffield Hallam University, Sheffield, S1 1WB, U.K.

In addition to analgesics and non steroidal anti-inflammatory drugs (NSAIDs), the established therapeutic management for rheumatoid arthritis (RA) is with orally-administered disease modifying anti-arthritis drugs (DMARDs) and the biologics especially anti-TNF agents (Saag *et al* 2008). However from the 1930s to 1990s injectable gold complexes in conjunction with NSAIDs were the standard therapeutic management for RA. Gold sodium thiomalate (GST) was the most commonly used and had a low cost. Several oral DMARDs such as sulphasalazine, D-penicillamine, hydroxychloroquine and ultimately methotrexate (MTX) were introduced for RA treatment over the 40 years from the 1940s. By the early 1990s the use intramuscular (IM) GST had markedly diminished most likely due to the reported functional and disease modifying benefits of low dose oral MTX alone or in combination with other DMARDs. In addition to MTX, over the last 10 years, the so-called biologic agents have been established as a significantly effective treatment for RA and early treatment intervention has been advocated (Saag *et al.* 2008). Most rheumatologists who have become consultants in the last 15 years have no experience with GST use. There has recently been a resurgence of interest in the pharmacology of GST and other gold complexes (Kean and Kean, 2008; Graham *et al.*, 2008; Wood *et al.*, 2008; Whitehouse, 2008) thus raising the possibility that there may be better understanding of their therapeutic actions and potential applications.

Rau (2005) has challenged the hypothesis of the superiority of MTX and that of the biologics in RA in a comprehensive comparison of GST with etanercept, adalimumab, anakinra, infliximab and MTX. Rau concluded that the oral DMARDs and the biologics were not necessarily better than GST and that a sufficient trial of DMARDs including GST should be used initially in RA treatment before the more expensive biologics (Rau, 2005). Support for the re-introduction of GST for RA were demonstrated in the 48 week

METGO RA trial (in 2005) on the efficacy and safety of adding IM GST to MTX for RA patients who had a sub-optimal response to 12 weeks or more of MTX use alone (Lehman *et al.*, 2005). At 48 weeks, there was an ACR 20 response in 61 % of MTX plus GST patients versus 30 % in the MTX plus placebo ($X^2=6.04$, $P=0.014$; logistic regression odds ratio=3.64 [95 % confidence interval 1.3, 10.4], $P=0.016$). Similar positive responses for MTX plus GST were observed in the ACR 50 and 70 assessments. Adverse events were minor. The authors concluded that the addition of the IM GST was both clinically and cost-effective for RA patients with a suboptimal response to MTX.

The American College of Rheumatology (ACR 2008) Recommendations for the use of DMARDs and biologics in RA (Saag *et al* 2008), state that biologics have been shown to be effective, but their benefit, like the DMARDs, is not sustained long-term. They also raised the major issue of concern with regards to some DMARDs (not GST) and all biologics with respect to the risk of exacerbating or facilitating infection. There is no risk for administration of GST to RA patients during infection, nor risk of facilitating an infection. The METGO study (Lehman *et al.*, 2005) was cited in the ACR references but not discussed in the text, and recommendations for IM GST use in RA were absent. There is current concern that patients in the USA who have limited or no health insurance are essentially denied access to biologics. In the UK the National Institute for Health and Clinical Excellence (NICE) issued a recent guidance (NICE, 2008) that after a failure on a first DMARD and a biologic that NICE do not support the use of a 2nd biologic except in a clinical trial. The NICE committee makes no mention of the METGO paper and no other recommendations for the use of GST.

Thus, for different reasons patients in the USA and UK, who have failed MTX or a biologic, or have a biologic “infection risk” may have limited access to further agents except oral DMARDs, but not GST. We advocate that the use of MTX + GST is an effective and cost effective treatment option for RA patients who have failed MTX or other oral DMARD combinations, or failed biologics, or are denied access to biologics.

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S5

Revisiting the conditional concept for pathology, pharmacology and toxicology

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This concept reminds us that ambient factors (or precise experimental conditions) may determine both (i) incidence/severity of inflammatory disease and (ii) affect the efficacy and toxicity of a drug according to the inflammatory 'context'.

Four examples will illustrate this:

1. The conditional concept was originally promulgated to explain how the efficacy of an NSAID to treat acute inflammation might be determined or amplified by endogenous "hormones" e.g. prostanoids, to express a constructive synergy (Whitehouse & Vernon-Roberts 1991) i.e. that disease could induce (or promote) drug action. Drug behaviour in pathogenic states such as chronic inflammation in rats is also modified by inhibition of hepatic drug disposition, effectively extending the "normal" half-life and perhaps lowering the toxicity threshold.
2. This aspect of a drug's toxicity being conditioned by disease is further illustrated by the gastric damage caused by paracetamol in rats suffering inflammatory stress (Rainsford & Whitehouse 2006; Whitehouse *et al* 2008). This analgesic is however non-gastrotoxic when non-inflammatory stressors are imposed (gastric hyperacidity, fasting or cholinergic stimulation).
3. Prostanoid auto-regulation of inflammation and induction of tolerance to arthritogens can both be compromised by simultaneous NSAID therapy in animals with arthritis. (Haynes *et al* 1990, Whitehouse *et al* 2008).
4. The paradoxical properties of the thiocyanate anion (SCN⁻) generated by hepatic detoxication of the hydrogen cyanide ingested from cigarette smoke or dietary cyanogens. By itself, thiocyanate supplementation in the drinking water amplifies the expression of chronic arthritis in rats induced with an environmental trigger (mycobacteria) endogenous auto-antigen (collagen type-II) or synthetic chemicals (avidine) i.e. the SCN⁻ supplement behaves here as a pro- or co-arthritis. Yet when SCN⁻ is given to rats developing arthritis together with gold or silver formulations that are not anti-arthritic per se, the combination of metal drug plus thiocyanate suppresses arthritis developments i.e. the SCN⁻ induces beneficial drug action (Whitehouse & Vernon-Roberts 1994).

This last example of ambivalent efficacy (both pro- and contra-arthritic) according to circumstance illustrates how non-genetic factors may determine outcomes of drug therapy for inflammatory disease, often unpredictably and sometimes inexplicably.

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S6

Pharmacological studies *in vitro*: problems in correlating concentrations with therapeutic drug concentrations

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A principle of clinical pharmacology is that the effects of drugs are related to their concentrations in plasma (Pratt, 1974). If the drugs are bound reversibly to plasma proteins, as many are, then a second principle is that the effects of drugs are related to the unbound concentrations (Graham *et al.*, 1999; Graham and Scott, 2003; Pratt, 1974). All too often, papers on drug effects *in vitro* contain claims about the mechanism of action of a drug when the studies were conducted at drug concentrations that are far greater than the plasma concentrations achieved by therapeutic dosage. Furthermore, many *in vitro* pharmacological studies are conducted in media containing little or no added plasma proteins, conditions in which the fraction of unbound drug is present at higher concentrations than *in vivo* (de Vries *et al.*, 1986; Szebeni and Weinstein, 1991). This is not considered widely. It is therefore probable that many drug effects that have been observed in studies *in vitro* do not occur during therapeutic dosage.

The actions of the NSAIDs including the newer selective COX-2 inhibitors have been studied widely and provide examples where these basic principles have often not been considered. Of 17 randomly-selected research papers on celecoxib published in 2008, 8 papers contained some consideration of the total therapeutic plasma concentrations (bound + unbound). There was no discussion of the unbound levels in any paper.

We are not recommending that all findings at supra-therapeutic concentrations should be rejected automatically. Supra-therapeutic concentrations of drugs may be required *in vitro* in order to produce an effect. This is particularly the case when the drug is a competitive inhibitor and high concentrations of the agonist or enzyme substrate are present. Possible metabolism of a drug *in vitro* should also be considered. More sophisticated and considered applications of the

principles of pharmacology should be applied to the interpretation of drug effects from *in vitro* studies.

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S7

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 are no specific preventatives approved for AD and the approved treatments provide time-limited symptomatic relief. Much evidence points to a crucial role for the amyloid precursor protein (APP) in the pathogenesis of AD encapsulated in the amyloid cascade hypothesis, i.e. excision of amyloid peptides by selective proteolysis of APP leads to accumulation and extracellular deposition in senile plaques in the brain, leading eventually to neurodegeneration (Hardy, 1997). It is generally held that neurofibrillary tangles (NFT), the other pathological hallmark of AD, are the result of the neurodegeneration rather than the cause. NFTs are composed of hyperphosphorylated and ubiquitinated tau. However, reports from a recent clinical trial have raised again the possibility that tau deposition may still be important, at least in the early stages of the disease (Wischnik *et al.*, 2008).

The key enzymes, secretases, involved in APP proteolysis have been identified and their suitability as targets for AD therapeutics has been explored (Osborne, 2008). Some secretases are involved in proteolysis of important cellular signaling pathways, so inhibitors of these enzymes may have undesirable side-effects. Epidemiological evidence has implicated inflammation in AD pathology and treatment with some anti-inflammatory drugs appears to reduce the risk for AD and/or delay the onset (Weiner & Frankel, 2006). Recent data however suggests that the effects of some of these drugs may be due to other actions, including a novel mechanism not yet seen with any other drug class (Kukar *et al.* 2008). Tarenflurbil (R-flurbiprofen) is a non-prostaglandin synthesis inhibitory NSAID that appears to modulate the activity of one of the key enzymes, γ -secretase, involved in amyloid

peptide excision, but does through binding to the substrate rather than the enzyme.

The implications of what is known about AD pathogenesis for prophylaxis and treatment of AD will be considered.

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S8

The carbonic anhydrases: potential therapeutic targets in renal cell carcinoma

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The zinc enzyme, carbonic anhydrase (CA), catalyses the reversible hydration of carbon dioxide to bicarbonate and a proton. Seemingly ubiquitous in nature, it is expressed in 15 different isoforms in humans, 12 of which are active isozymes. In addition to their long established roles in respiration and acid-base regulation, CA isozymes are now known to play crucial roles in many other fundamental physiological processes, including the biosynthesis of lipids and nucleotides. Whilst the rate of the uncatalyzed CA reaction may be sufficient to accommodate basal metabolism, it appears that CA activity is required to sustain the higher level of metabolic flux associated with enhanced levels of cell growth such as those encountered in cancer cells (for reviews see Chegwidden *et al.*, 2000; Chegwidden, 2006).

Renal cell carcinomas account for about 2% of all adult malignancies. They tend to metastasize before giving rise to local signs or symptoms, which are characteristically resistant to chemotherapy and radiotherapy. In the normal human kidney, CA II, which is among the most widespread and fastest of all enzymes, is expressed in the cytoplasm, CA VB in the mitochondrion, and CA IV and XII on the extra-cellular surface. The CA IX isozyme appears to be virtually specific to cancer cells and is strongly expressed on the cell surface of most renal cell carcinomas, where its presence is considered to be diagnostic. CA IX expression is down-regulated by pVHL and up-regulated by anoxia. It is possible that both, or either, the cytoplasmic and the extra-cellular CA isozymes may facilitate the extrusion of protons from the cell, to create a milieu that is more conducive to cell invasion and metastasis.

We investigated the effects of highly specific CA inhibitors on both the growth and invasive properties of renal cancer cells in culture, and on the growth of human tumors after

implantation into immunodeficient mice. We employed two human renal cancer cell lines: one which strongly expresses both the extra-cellular CA IX and XII isozymes, and one which expresses neither. CA inhibitors inhibited the growth and invasion of both cell lines. They also inhibited the growth rate of tumors derived from these human cell lines after implantation into immunodeficient mice.

Our data suggest that the development of specific inhibitors targeted at carbonic anhydrase isozymes may be of value in therapy for renal cell carcinoma.

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S9

Some actions of nitric oxide-donating NSAIDs on isolated preparations of vascular and other smooth muscles

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In Cambridge, third year natural science, pre-clinical medical or veterinary students have the opportunity to spend one term of original research. This important aspect of a young person's scientific training relies very heavily of the provision of worthwhile and exciting projects that, within the short time available for experiment, can still lead to publication in peer-reviewed journals. Such projects should not simply aim to teach manual dexterity and skill in some sophisticated, cutting-edge technique but offer every opportunity for the student to hone their intuitive and reasoning talents to unravel a puzzle, to which the student's research supervisor does not have an answer but has confidence that the project will work. One example of this bridge between didactic teaching and original research, undertaken in close collaboration with Kim Rainsford, concerns the ability of nitric oxide-donating non-steroidal anti-inflammatory agents (NO-NSAIDs) to affect the reactivity of isolated vascular and other smooth muscles.

Following from the observation by Tashima *et al.*, (2000) that nitroxybutyl aspirin (NCX-4016) could reduce the gastric irritancy of its parent aspirin it was decided to examine the ability of NO-NSAIDs to produce significant vasodilatation in isolated tissue preparations. Previous student investigations in our laboratory had established that ring segments of the common digital artery of the forefoot of the fallow deer

(*Dama dama*) would provide an ideal test preparation (Milton *et al.*, 1999). Rings from mixed breeds of sheep have also been employed. Furthermore, use has been made of the electrically-stimulated isolated anococcygeus muscles of the rat.

In the main, arteries were obtained from the forefeet of fallow deer of either sex (38–48 kg body weight) killed under E.U. red meat regulations. If kept at around 4°C in modified Krebs-Henseleit solution aerated with 95% O₂ and 5% CO₂, the vessels can be used for experiment, after cutting into rings of approx 3 mm in length, for at least 10 days after the animal is slaughtered. Moreover, because the animals are killed for their meat, the vessels are in relatively plentiful supply allowing statistically valid results to be obtained from large numbers of animals.

Preliminary experiments (Benedict *et al.*, 2005) showed that concentration-response curves were generated from the changes in isometric tension caused by the cumulative addition of 5-hydroxytryptamine (5-HT) in the presence and absence of aspirin and its analogues. Neither the presence of aspirin or of aspirin butyl ester at concentrations up to 10⁻⁴ M had any significant effect on the responses of the rings. However when the experiment was repeated in the presence of 10⁻⁴ M aspirin nitroxybutyl ester (NO-aspirin), the concentration-response curve to 5-HT was significantly shifted to the right (EC₅₀ of 5-HT, control: 9.01 ± 0.72 × 10⁻⁷ M, aspirin nitroxybutyl ester [10⁻⁴ M]: 5.20 ± 0.49 × 10⁻⁶ M, P < 0.001. While the addition of 2 ml of guinea pig blood to the bathing fluid had little effect on the action of 5-HT, it greatly reduced the effectiveness of NO-aspirin. Similar results were obtained when ovine arterial rings were used and when the nitroxybutyl esters of indomethacin and naproxen were tried.

The only anomalous results were that the difference in effect between ibuprofen and its nitroxybutyl ester was often reversed. An explanation for this is currently being sought. NO-aspirin also elicited a clear reduction in the responses to electrical stimulation of the rat anococcygeus muscle. In addition, evidence, from a parallel study in Sheffield Hallam University (Hayat Mohamed, 2004; Sandeep Pidakala, 2005, unpublished) showed that in the electrically stimulated anococcygeus muscle, the actions of NO-aspirin, reached its maximum effect after about 20 minutes of incubation, taking a further one hour or more to wear off. These results suggest that the smooth muscle relaxant effects of NO-aspirin may be due to release of NO from the nitrobutoxyl-ester. However, further experiments are required in which the relation between the effects of the NO-NSAIDs and the kinetics of NO release from the rat tissues under similar conditions employed in these studies.

What is clear, however, is that the isolated rings of the common digital arteries from the forefeet of the fallow deer and of the more readily available sheep, provide a simple, reliable and very durable preparation with which to study drugs that affect smooth muscle tone and response.

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S10

Why pain gets worse – molecular mechanisms of inflammatory heat pain

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One of the distinguishing characteristics of the sensation of pain is that it increases when a constant painful stimulus is applied. This process, known as sensitization or hyperalgesia, is caused by the release of pro-inflammatory mediators, amongst which are prostaglandin E₂, bradykinin and nerve growth factor (NGF). Heat pain is promoted by all three of these mediators, and also by a multitude of others (Huang *et al.*, 2006). At least three different intracellular signalling mechanisms are important in mediating the effects of these inflammatory mediators on the heat-sensitive ion channel, TRPV1. Bradykinin and PGE₂ enhance the probability that TRPV1 channels will be activated by a heat stimulus by promoting phosphorylation of TRPV1 by protein kinases C and A, respectively (Cesare & McNaughton, 1996; Cesare *et al.*, 1999; Bhave *et al.*, 2002). The main action of NGF is instead to increase the expression of TRPV1 channels in the neuronal cell membrane by promoting trafficking from a subcellular vesicle store (Zhang *et al.*, 2005). This process depends on phosphorylation of TRPV1 at a single tyrosine residue, Y200, by the non-receptor tyrosine kinase Src (Zhang *et al.*, 2005).

In more recent work we have found that phosphorylation of TRPV1 by PKC and PKA is critically dependent on a scaffolding protein, AKAP79, which binds PKA and PKC into a signalling complex together with TRPV1 (Zhang *et al.*, 2008). Preventing binding of AKAP79 to TRPV1 completely ablates sensitization by pro-inflammatory mediators acting via PKA and PKC. AKAP79 is therefore a final common element in heat hyperalgesia, on which the effects of multiple proinflammatory mediators converge. The dependence of sensitization of TRPV1 on AKAP79 raises the possibility that disrupting binding may reverse heat hyperalgesia *in vivo*. The binding site of AKAP79 to TRPV1 may therefore prove to be an attractive target for the development of novel analgesics.

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S11

Analgesic Targets: Today and Tomorrow

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Paracetamol, opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used as analgesics for the symptomatic control of pain. Today, we understand substantially more about the mechanism(s) of action of these agents both peripherally and centrally. Recent advances in neuroscience, however, have led to an enhanced understanding of pain pathways and the consequent unmasking of several novel targets for new analgesics (Dray 2008).

In injured tissue, acute pain is evoked locally via the action of cellular components of the inflammatory process, prominently the prostanoid products of COX-2 enzyme up-regulation at specific sites, bradykinin, cytokines and nerve growth factor(s). The prostanoid most implicated is prostaglandin (PG) E₂. PGE₂ signals pain input by binding to receptors that regulate calcium and sodium channels on nociceptors, reducing their threshold for stimulation thus enhancing their sensitivity to noxious stimuli. Inflammation in the periphery generates pain hypersensitivity in part by a process of spinal sensitization (the wind-up phenomenon) in which COX-2-derived PGE₂ interferes with the synaptic inhibitory effect of glycine and GABA in the dorsal horn of the spinal cord (Reinold *et al.*, 2005). Given our current understanding it is clear that for effective control of pain, non-steroidal anti-inflammatory drugs, including the COX-2-selective inhibitors, must possess both a peripheral and central mechanism of action. Clearly, for optimal efficacy their penetration into the brain is a necessity. Inhibiting central COX-2 activity greatly reduces inflammatory pain hypersensitivity while in the periphery it very effectively combats the local inflammatory prostanoid-dependent processes.

In terms of novel targets for analgesics of the future those that are attracting some research attention are bradykinin, certain adenosine receptors, the TRPV₁ receptor, the TTX-resistant sodium channel (Nav_{1.8/1.9}) (Julius and Basbaum, 2001), presynaptic neuronal calcium channels (for example Ca_v2.2) (Winquist *et al.*, 2005), brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) receptors (Coull *et al.*, 2005; Torsney and MacDermott, 2005). Bradykinin exerts its effects via activation of B₁ and/or B₂ receptors. The B₁ receptor is normally silent but is up-regulated when inflammation is initiated. In contrast the B₂ receptor is constitutively present in certain tissues. At peripheral sites of inflammation, bradykinin sensitizes nociceptors to activating stimuli via modulation of the TRPV₁ ion channel (Julius and Basbaum, 2001). Centrally, its mechanism of action is as a neuromodulator at the level of the spinal cord where it augments the release of the fast-acting neurotransmitter glutamate (Wang *et al.*, 2005). It is likely that this effect is consequent upon up-regulation of a use-dependent, pre-synaptic N-type Ca channel (Ca_v2.2). Additionally, bradykinin

enhances activation of both AMPA and NMDA receptors by glutamate (Wang et al., 2005). Brain-derived neurotrophic factor is released from microglia in response to adenosine receptor (P2X₄) activation. As it is currently envisaged, BDNF acts through TrkB receptors to reverse the inhibitory signaling action of glycinergic and GABA-ergic interneurons (Coull et al., 2005; Torsney and MacDermott, 2005). This is achieved by depolarization of post-synaptic cell bodies of the second order sensory neurons in the dorsal horn, via facilitation of chloride ion efflux. The excitement surrounding this latter observation is that the BDNF/TrkB mechanism has all the hallmarks of a key event involved in the establishment of neuropathic pain syndromes (Torsney and MacDermott, 2005). Thus, the unraveling of some of these newer mechanisms underlying pain signaling is a cause for significant optimism that novel, effective analgesics are a future reality for clinical practice.

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S12

The janus faces of migraine

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The presence of an inflammatory component in migraine is now generally accepted, the evidence including the clinical improvement with anti-inflammatory medication (aspirin, NSAIDS, prednisone) and the increased levels of vasoactive neuropeptide, CGRP, in the jugular venous blood during an attack (Waeber & Moskowitz, 2005). One favoured hypothesis holds that inflammatory mediators, such as CGRP, acting on trigeminal afferent nerve fibers in the meninges, induce hypersensitivity of peripheral and central trigeminal neurons; the pulsation of intracranial arteries becomes painful and, in addition, the regulation of pain pathways to the thalamus is disturbed. Through an extended study of a patient with an extremely complex form of migraine (McComas, 2006), we now present evidence that, in at least some migraine patients, any inflammatory events are probably secondary, and that the headache, together with the aura and other migraine symptoms, is generated in the cerebral cortex.

Patient KB is now 74 yrs old. A former nurse, she started to have episodes of sudden vertigo and tinnitus, followed by excruciating occipital headache, nausea and vomiting at age 21; basilar migraine was eventually diagnosed. At 48 she developed trigeminal neuralgia in her right lower jaw

and underwent radiofrequency surgery of the Gasserian ganglion, with temporary remission. Subsequently, however, spontaneous pains would develop in various parts of the body, mostly on the right side, and at age 64 the attacks became associated with transient right-sided hemiplegia. A diagnosis of hemiplegic migraine was made and an excellent response obtained with acetazolamide. At age 68 the attacks of pain and paralysis returned, and were more severe. There was now quadriplegia, followed by coma and respiratory arrest, each episode lasting approximately two hours. With two or three such attacks occurring every day, and with no response to drug treatment, transcranial magnetic stimulation (TMS) was tried as a last resort. The results in this and later attacks were dramatic, the pain and paralysis resolving abruptly after shocks applied over the contralateral somatosensory and motor areas of the cortex. Similarly impressive results have since been obtained in a patient with ophthalmoplegic migraine, and there are now three controlled studies showing the benefit of TMS in migraine, especially in those with visual auras. The efficacy of stimuli applied to the cortex indicates that the latter, not the brainstem, is the generator of migraine symptoms. Further, the near-instantaneous results make a causative role for inflammation unlikely.

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S13

Treating osteoarthritis in 2008: evidence, expert opinion and commonsense

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Osteoarthritis (OA) is the most common type of arthritis and an important cause of chronic musculo-skeletal pain and mobility disability in elderly populations worldwide. Knee and hip pain are the major causes of difficulty in walking and climbing stairs in the elderly and as many as 40% of those over the age of 65 in some European communities suffer symptoms associated with knee or hip OA.

More than 50 modalities of non-pharmacological, pharmacological and surgical therapy for knee and hip OA are described in the medical literature but there can be few areas of medicine where there is a greater need for sensibly balanced, impartial, evidence-based advice to assist clinicians in choosing optimal treatments for individual patients. Guidelines for the treatment of OA have been previously

criticised for poor applicability and inadequate stakeholder involvement as well as for lack of methodological rigour.

The Osteoarthritis Research Society International (OARSI) has recently published global evidence based consensus treatment guidelines for osteoarthritis (OA) of the hip and knee (Zhang *et al.* 2008) following a critical appraisal of 23 existing guidelines and a systematic review (SR) of the evidence for relevant therapies from 2002–2006 (Zhang *et al.* 2007). Twenty five carefully worded recommendations were generated by consensus by 16 experts from 4 medical disciplines (primary care, rheumatology, orthopaedics and evidence-based medicine), 2 continents and 6 countries (Canada, USA, UK, France, Netherlands and Sweden). The guidelines included recommendations for a core set of 20 modalities of therapy including (6 pharmacological, 8 non-pharmacological and 5 surgical) plus the combination of pharmacological and non-pharmacological therapies which are currently recommended in existing guidelines. A considerable number of new studies and SRs have been published in the last 2 years. To appraise the quality of the OARSI guidelines and to assess whether recent evidence should alter recommendations for core therapies for OA the quality of guidelines was assessed using the AGREE (Appraisal of Guidelines for Research and Evaluation) instrument for scope, stakeholder involvement, rigour, clarity, applicability and editorial independence, and overall quality. Assessments were undertaken by 2 panels of independent international experts from a variety of health professional disciplines. The systematic review included all new guidelines, SRs, randomised controlled trials (RCTs) and economic evaluations (EEs) published between 31 January 2006 and 31 January 2008. The quality of the RCTs included in the SRs and of others retrieved from the literature search were appraised, and where possible effect size (ES), number needed to treat (NNT), relative risks (RR) or odds ratio (OR) and cost per quality adjusted life years (QALY) gained were estimated. Sensitivity analysis and cumulative meta-analysis were conducted to examine the impact of studies published after 2006 and the stability of the effect.

The OARSI guidelines had higher quality scores than previously published guidelines but scores for stakeholder involvement and applicability remained low. Out of 1347 citations from 2006–2008, 2 guidelines, 57 SRs, 200 RCTs and 16 EEs met inclusion criteria. Core therapies, (treatments supported by meta analyses or RCTs and recommendation by all guidelines addressing that therapy), remained unchanged. These included exercise, education, self-management, acetaminophen and COX-2 selective or non-selective NSAIDs with PPI. ES changed with inclusion of additional trials. Cumulative meta-analysis indicated stability of efficacy for some therapies (eg, NSAIDs) but not for others (eg, glucosamine and chondroitin sulphate).

In conclusion, the quality of the OARSI guidelines, though better than previous guidelines, could be further improved by wider stakeholder involvement and greater attention to applicability. Recently published evidence has resulted in changes in the calculated risk-benefit ratio for some treatments for osteoarthritis. The rapid increase of new evidence presents challenges to clinicians and guideline developers. A regularly updated, evidence-based osteoarthritis research database of well characterised trials of all modalities of treatment for OA would be very useful.

A number of important caveats concerning reliance on evidence hierarchies do, however, remain. Do meta-analyses really really always provide better evidence than good quality RCTs and which meta-analysis should one trust when several have been undertaken? Attention needs to be paid to the criteria and quality of the included studies, to comorbidities, co-interventions, and the ever present possibility of patient selection bias, to variability in outcome reporting, heterogeneity of outcomes and to publication bias. Great care will always be required before trustworthy comparisons of effect sizes across interventions can be made. RCTs may not always provide better evidence than observational studies and the efficacy of surgical procedures must not be downgraded because RCTs are not appropriate. In the last analysis recommendations for any therapy must involve careful consideration of the balance between potential harms and benefits by patients as well as by their physicians. Many of these concerns about the limitations and clinical usefulness of evidence-based medicine were raised and elegantly discussed by Watson Buchanan at the turn of this century (Buchanan and Kean 2001).

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S14

Modifying cardiovascular risk in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a systemic autoimmune condition. Although synovitis is the most obvious manifestation of aberrant inflammation, cardiovascular disease is responsible for most of the premature mortality as well as significant morbidity in this inflammatory disease (Hall and Dalbeth, 2005; Tier *et al.*, 2008). Recently, it has been realised that drugs which rigorously control the inflammatory disease reduce cardiovascular risk and that HMG CoA reductase inhibitors (statins), which are usually prescribed to reduce cholesterol, also reduce synovitis in RA (Dalbeth *et al.*, 2005; Hall and Dalbeth, 2005). Angiotensin II has been shown to have proinflammatory properties, in addition to its vasoactive effects. This raised the possibility that angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) would also reduce synovitis.

The effects of ACEI and ARB were studied in the collagen-induced model of inflammatory arthritis in mice. Mice received oral quinapril (10 mg/kg/day) or candesartan (5 mg/kg/day) at the time of arthritis induction (prophylaxis protocol) or at the onset of mild arthritis (therapeutic protocol).

Concentrations of immunoglobulin G (IgG) subtypes specific for bovine type II collagen and TNF α were measured by enzyme-linked immunoassay. Quinapril significantly decreased the severity of inflammatory arthritis when given prophylactically ($p < 0.001$) or therapeutically ($p < 0.01$). Antigen-specific IgG2a antibodies were reduced by 52% ($p < 0.05$) in the quinapril prophylaxis protocol and articular expression of TNF- α was reduced by 68% ($p = 0.01$). ACE inhibition suppressed LPS-stimulated production of TNF- α by human monocytes in vitro. Parallel experiments with the candesartan yielded similar results.

A Trial in Rheumatoid Arthritis of LISinopril (TRALIS) is currently underway. The primary outcome measure is the disease activity score (DAS)-28 but vascular outcome measures have also been included. Since cardiovascular risk reduction should be integrated with the management of synovial disease activity in RA, we published a cardiovascular risk reduction algorithm and have recently audited East Anglian practice against this (Teir *et al.*, 2008). The audit data demonstrate suboptimal risk management and highlight several challenges involved in the implementation of improved therapeutic strategies to the clinic.

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S15

The many talents of hydroxychloroquine

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Hydroxychloroquine (HCQ) is a 4-aminoquinolone drug that has been used as an antimalarial drug for many years. However, HCQ is also a potent anti-inflammatory agent and its benefit in helping to control a variety of autoimmune diseases is well known. HCQ is particularly useful for managing patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Even though it was stated for many years that HCQ was not particularly useful for managing the major organ involvement that can occur in SLE, recent publications have demonstrated that the morbidity and the mortality of SLE are improved in SLE patients who are treated with HCQ (Ruiz-Irastorza *et al.*, 2006). Discontinuing this drug produces flares of disease activity in lupus patients (The Canada Hydroxychloroquine Study Group, 1991) and despite the fact that HCQ crosses the placenta, it has been shown by us and other investigators to be safe during pregnancy, particularly in mothers with SLE. The current standard of care for managing

pregnant patients with SLE is to continue the lowest possible dose of HCQ throughout pregnancy (Ostensen *et al.*, 2006), as lupus disease activity puts both the mother and the fetus at risk for adverse outcomes. The anti-inflammatory effects of HCQ can be attributed to a variety of mechanisms, including: inhibiting the release of lysosomal enzymes, interfering with antigen processing by monocytes and macrophages, decreasing class II MHC mediated interaction between antigen presenting cells and T cells, decreasing the secretion of inflammatory cytokines and reduced the synthesis of prostaglandins and leukotrienes. Hydroxychloroquine inhibits toll-like receptor signaling and has also been shown to potentiate Fas-mediated apoptosis of rheumatoid synoviocytes. Other benefits of HCQ include its antiviral effects, the ability to lower lipids and its ability to interfere with platelet aggregation, making it a very useful agent for patients with phospholipid antibody syndrome. Despite these many diverse effects, the only FDA approved indications, apart from its antimalarial use, are SLE and rheumatoid arthritis, but this drug is used off label with significant success in many other conditions including autoimmune, prothrombotic, granulomatous and dermatological diseases.

This review will discuss the many benefits of HCQ, its mechanisms of action and factors that influence its efficacy and toxicity. This very versatile drug warrants additional pharmacological and clinical study so that it may be used to its full potential.

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S16

PK/PD integration and PK/PD modelling of nsaid for dosage determination in the cat

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NSAIDs are used extensively for the relief of pain and inflammation in the cat, for example perioperatively and for the control of chronic pain in osteoarthritis. Pre-clinical studies

to determine dosage schedules for subsequent evaluation in clinical trials may be based on the conventional dose titration approach but this has several disadvantages and limitations. As an alternative, pharmacokinetic-pharmacodynamic (PK-PD) integration and PK-PD modelling may be used (Toutain, 2002). The dose over a dosing interval is determined from the relationship $\text{Dose} = \text{Total clearance} \times \text{Target concentration/Bioavailability}$. Clearance and bioavailability are determined, in the first instance in healthy animals, from plasma concentration-time profiles after dosing intravenously and by the intended clinical route (usually oral or subcutaneous injection for NSAIDs in the cat). Studies in our laboratory have used 3 approaches to establish the target concentration and corresponding dosages: (a) in whole blood *in vitro* assays NSAID potency (IC_{50} and IC_{80}) for inhibition of the COX-2 isoform of cyclooxygenase is determined; (b) in a tissue cage model of acute inflammation, based on the intracaveal injection of the mild irritant carrageenan, the time courses of drug penetration into exudate and inhibition of prostaglandin (PGE_2) are determined; and (c) in a model of acute paw inflammation induced by subcutaneous injection of kaolin, biomarkers and surrogates for clinical endpoints (e.g. pain and lameness scores, skin temperature rise) are determined.

Based on whole blood assays of COX inhibition, once daily dosages have been determined for meloxicam (preferential for COX-2 in the cat), carprofen (selective for COX-2) and robenacoxib (highly selective for COX-2). Robenacoxib has been developed for use in companion animal medicine (King et al., 2008). IC_{50} COX-1:COX-2 ratios were 3.05, 25.6 and 502, respectively, whilst corresponding IC_{80} COX-1:COX-2 ratios were 21.4, 64.9 and 478 (Giraudel et al 2005a, 2008a). Based on *in vitro/in vivo* extrapolations and an IC_{50} for COX-2 inhibition as the lowest target concentration to be achieved for clinical efficacy, predicted daily doses (mg/kg) were 0.11 (meloxicam) 0.21 (S-carprofen) and 0.27 (robenacoxib). To achieve good clinical responses, it is likely that a higher level of COX-2 inhibition will be required; for IC_{80} corresponding doses (mg/kg) were 0.17, 0.32 and 1.53. Doses recommended by manufacturers are 0.05–0.3 (meloxicam), 2.0 (S-carprofen) and 1–2 (robenacoxib) mg/kg, respectively.

In a tissue cage model of inflammation the blood clearance of robenacoxib was relatively rapid (0.54 L/h/kg) and elimination half-life was relatively short (2.1 h) but elimination half-life from exudate was approximately 24 h and inhibition of synthesis of PGE_2 in exudate was significant up to 24 h, indicating a likely duration of action in clinical subjects longer than would be predicted from blood clearance of the drug. PK-PD modelling for meloxicam and robenacoxib, using indirect response models, has been applied to data obtained in the kaolin paw inflammation model (Giraudel et al, 2005b, 2008b). For pain score, lameness score and skin temperature difference IC_{50} values (ng/mL) for plasma concentration of meloxicam were 883 ± 215 , 911 ± 189 and 1298 ± 449 , respectively and corresponding values for blood concentration of robenacoxib were 112 ± 55 , 39 ± 26 and 168 ± 114 . An advantage of PK-PD modelling is that, once the whole of the concentration-effect relationship is established (this can sometimes be achieved by testing only a single dose in a single group of animals), computer simulations may be

undertaken to determine drug response profiles for any dosage regimen. For example, a dose of 0.3 mg/kg meloxicam is predicted to provide 51 % of the maximum drug response on pain, whilst a dose of 0.1 mg/kg is sub-threshold, providing only 0.02 % of maximum response. A dose of 2 mg/kg robenacoxib is predicted to achieve 55 % of the maximum drug response on lameness.

These data have been used to select a dose rate and dosing interval for robenacoxib in the cat for subsequent evaluation in clinical trials, designed to establish the relief of pain and inflammation associated with surgery and the chronic pain of osteoarthritis. The clinical findings have established good efficacy with once daily dosage of 1–2 mg/kg. In these trials population PK data have shown that robenacoxib clearance is slower and mean residence time is longer than corresponding values obtained in pre-clinical studies. We conclude that pre-clinical PK and PD data provide an invaluable basis for initial dosage determination and that population PK data in clinical subjects are also required to optimise dosages of NSAIDs such as robenacoxib.

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S17

Dual action of glucocorticoid hormones on the gastric mucosa: how the gastroprotective action can be transformed to ulcerogenic one?

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Gastric ulceration and glucocorticoid hormones have been discussed in many contexts. Various types of gastric damage (acute stress erosions vs. peptic ulcer disease) and situations with altered glucocorticoid hormone supply (acute and chronic stress-induced secretion, acute and chronic treatment of patients or experimental animals with synthetic compounds) have been considered. Although there is a long-standing debate over whether glucocorticoid therapy leads to peptic ulcer disease in human it is established that administration of glucocorticoids to experimental animals can result in an acute gastric erosion formation. In some cases administration of glucocorticoids to animals can attenuate

gastric erosion formation. It is also known that basal glucocorticoid production contribute to the maintenance of the gastric mucosal integrity. The most controversial question is the question about the action of stress-induced glucocorticoid production. The ulcerogenic properties of exogenous glucocorticoids were extrapolated to the properties of endogenous glucocorticoids produced in stress situations and it has been generally accepted for several decades that glucocorticoids released during stress caused an ulcerogenic response in the stomach. As this view is difficult to reconcile with the adaptive role of glucocorticoids, we designed experiments to clarify the validity of this dogma. The results obtained do not support the traditional paradigm and suggest that glucocorticoids released during acute activation of the hypothalamic-pituitary-adrenocortical (HPA) axis are important gastroprotective factors (Filaretova et al., 1998, 2002). We demonstrated that the glucocorticoids may contribute to gastroprotection by maintaining local gastric mucosal and general body homeostasis, including carbohydrate homeostasis (Filaretova et al., 2007). Thus, beneficial action of glucocorticoids released during acute activation of HPA axis on the stomach are opposite to the harmful actions of exogenous glucocorticoids used at pharmacological doses. How physiological gastroprotective action can be transformed to pathological ulcerogenic effect? We hypothesized that glucocorticoid-induced disturbance of carbohydrate regulation may be responsible for the transformation. The results obtained support the hypothesis and suggest that maintenance of blood glucose levels may be responsible for the gastroprotective action of glucocorticoids, while disturbance of carbohydrate regulation during glucocorticoid-induced hyperglycemia (accompanied with the signs of catabolic effects) may account at least partly for the ulcerogenic action of glucocorticoid hormones.

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S18

Pharmacological analysis of 2-adrenoceptor subtypes mediating analgesic, anti-inflammatory and gastroprotective actions

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Different α_2 -adrenoceptor subtypes were identified ($\alpha_{2A/D}$, α_{2B} , α_{2C}) on the basis of pharmacological and molecular cloning evidence (Bylund, 1988; Hein & Kobilka, 1995). $\alpha_{2A/D}$ -adrenoceptors display high affinity for oxymetazoline and low affinity to prazosin and ARC 239, whereas converse selectivity for these agents is displayed by $\alpha_{2B/2C}$ -adrenoceptors. Increasing number of evidence suggest the functional role of different α_2 -adrenoceptor subtypes. $\alpha_{2A/D}$ -adrenoceptors were shown to mediate the central hypotensive response as well as the sedative effects of α_2 -adrenoceptor stimulants. Both α_{2A} and α_{2B} -adrenoceptor subtypes were involved in antinociceptive action. Activation of pre-synaptic α_2 -adrenergic receptors has been known also to modulate several responses in gastrointestinal tract, like gastric acid secretion, gastrointestinal motility, gastric emptying and gastric mucosal protection (Gyires et al., 2000; Müllner et al., 2002; Fülöp et al., 2005).

The aim of experiments was to analyse which α_2 -adrenoceptor subtypes may mediate the gastroprotective, anti-inflammatory and antihyperalgesic actions of α_2 -adrenoceptor stimulants.

Experiments were performed in male Wistar rats. Gastric mucosal lesions were induced by indomethacin (20 mg/kg p.o.) and 100 % acidified ethanol. Gastric motility was measured in vivo in anaesthetised rats using the balloon method. Acute inflammation was induced by intraplantar injection of 1 % carrageenan and carrageenan-induced hyperalgesia was measured in Randall Selitto-test. The drugs were given orally (p.o.), intravenously (i.v.) and intracerebroventricularly (i.c.v.). Also the expression of α_2 -adrenoceptor subtypes in gastric mucosa of the rat was determined by RT-PCR.

Results: 1. Clonidine and rilmenidine inhibited the ethanol- and indomethacin-induced gastric lesions in the dose range of 13–94 nmol/kg orally. The effect was reversed by yohimbine (2 mg/kg s.c.) and prazosin (0.1 mg/kg s.c.). 2. Gastric motor activity was inhibited by clonidine in higher dose-range (1.3–3.8 μ mol/kg i.v.), the effect was reversed by yohimbine but not by prazosin. 3. Clonidine and rilmenidine exerted pronounced anti-inflammatory action in carrageenan-edema test in the gastroprotective dose range, the anti-oedematous effect was reduced by yohimbine but not by prazosin. 4. Similarly, clonidine and rilmenidine inhibited the carrageenan-induced hyperalgesia in Randall-Selitto test also in the gastroprotective dose-range, however, this effect was reversed by both yohimbine and prazosin. 5. Expression of α_{2B} and α_{2C} -adrenoceptor subtypes in gastric mucosa was much lower, than that of α_{2A} subtypes.

Conclusion: α_2 -adrenoceptor stimulants, clonidine and rilmenidine induced gastroprotective, anti-inflammatory and antihyperalgesic action in the same dose range, while inhibition of gastric motility was induced in higher doses. α_{2B} -like adrenoceptor subtypes may mediate the gastroprotective action, where central component is likely to be essential. Similarly, α_{2B} -adrenoceptor subtypes may also be involved in the antihyperalgesic action, however, α_{2B} adrenoceptor subtypes may not play a role in the anti-inflammatory action.

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S19

Protection against NSAID-related adverse reactions by multiple stratagems

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Serious adverse reactions (ADRs) have been associated with the non-steroidal anti-inflammatory drugs (NSAIDs) since the introduction of many of them in the 1970s (Rainsford 1997; Rainsford & Velo, 1992). Originally, most concerns involved gastro-intestinal (GI) ulcers and bleeding (PUBs). Subsequently many studies in experimental animals and clinico-epidemiological studies were undertaken to identify those drugs with a higher risk of causing GI ulcers together with patient related risk factors (Kean *et al.*, 2008; Rainsford, 1997, 2001; Rainsford *et al.*, 2008). Extensive research over the past 3–4 decades has defined the mechanisms of GI, renal and hepatic injury and although some aspects of this research are arguably incomplete there is considerable understanding of the major factors involved in the development of these side effects in the major organ systems (Rainsford, 1997, 2001, Rainsford *et al.*, 2008). Likewise, much information has been gathered on the pharmacokinetics (PK) of the NSAIDs, the potential for drug interactions (of which there are many) and patient-related (disease) factors relating PK and pharmaco- or toxico-dynamics (PD) to development of ADRs. There are over 200 commonly used drugs and natural products which are heavily dependant upon liver metabolism for detoxification. With the liver being a major site for detoxifying NSAIDs, serious issues arise concerning the drug burden on the detoxification by the liver when combinations of NSAIDs with other drugs or nutraceuticals are taken concurrently.

The striking thing is that very little of this extensive information has been effectively translated into clinical practice and in the commercial management of the NSAIDs. Even the elegant molecular biological research which led to the development of the newer class of highly selective COX-2 inhibitors (coxibs) has had limited, if somewhat controversial impact on clinical practice (Kean *et al.*, 2008; Rainsford, 2001; Rainsford *et al.*, 2008).

Much of the problem has to do with the commercial and clinical management of these drugs. They have been and are still excessively promoted such that physicians believe they are safe and effective without clear caveats for limitations

of use and minimal exposure (dosage) and the enormous potential for drug interactions, especially in the liver. The evidence exists for these factors especially interactions with commonly used drugs (e.g. statins, anti-diabetic agents, diuretics, anti-hypertensive agents) but this is not translated into practice. The strategies that involve reliance of proton pump inhibitors (PPIs) for use in preventing PUBs in the elderly at risk have problematic outcomes and are expensive solutions. It is doubtful if PPIs are good for the nutritional state of these elderly patients who already have reduced gastric functions (Kean *et al.*, 2008; Rainsford *et al.*, 2008). The use of misoprostol for reducing GI ADRs has been limited by the frequent occurrence of diarrhoea from this drug. Yet it has not been appreciated that since there is one active (SC-30249) as well as three inactive isomers of misoprostol, the occurrence of diarrhoea can be avoided by using the active isomer linked to a polymer while at the same time retaining GI mucosal protective properties (Perkins *et al.*, 1994).

The development of nitric-oxide donating NSAIDs (NOx-NSAIDs) could have been, and indeed by some was, predicted to have limited value for prevention of GI ulcers. This is related to the rapid metabolism of the nitro-esters combined with the short half-life of NO resulting in very limited mucosal protection with time after administration of NOx-NSAIDs (e.g. NO-naproxen). However, the side benefit has been that some of these drugs could be useful in patients at risk of cardio-vascular (CV) conditions.

There have been drugs that were selected for clinical development that could have easily been predicted as being associated with one or more serious ADRs. Thus, the liver injury with lumiracoxib could have been predicted from its metabolism (Kean *et al.*, 2008). Even the PK and mechanisms of action of rofecoxib could have predicted its CV and renal ADRs. More conservative management following the introduction of this and some of the other coxibs could have enabled a period of sensible appraisal, which with good information and clinical guidance of what had been identified as risk factors could have prevented the disaster that has befallen rofecoxib and many other NSAIDs.

Recently, the recognition that CV along with the hepatorenal and GI ADRs of NSAIDs raises the issue that there are considerable risks from intake of NSAIDs by patients with rheumatic conditions (Kean *et al.*, 2008). Thus, simple identification of those NSAIDs at higher or lower risks for developing serious or even non-serious ADRs is more complex than envisaged years ago. In attempts to gain an overall assessment of the risks associated with NSAID use we have formulated a composite assessment or algorithm, termed a “TRIAD toxicity” [TT] profile from data on ADRs attributed to NSAIDs, as well as paracetamol, in the GI, hepatic, cardio-renal and cardiovascular systems. This algorithm was devised by summing the published data on the relative risks of 10 frequently prescribed NSAIDs (including coxibs) as well as paracetamol ranked according to their GI, CV and hepato-renal toxicities. The data showed that some NSAIDs (e.g. ibuprofen, including coxibs (celecoxib, etoricoxib) and paracetamol had, overall, lower risks of severe ADRs compared with more widely used NSAIDs (e.g. diclofenac, naproxen) and lumiracoxib. This approach could provide a composite view of the relative incidence of overall toxicity associated with NSAIDs to help make a more informed se-

lection of particular drugs for patients at risk of developing adverse reactions in different organ systems.

In conclusion, it is suggested that a comprehensive understanding of the risks of major organ toxicity combined with development of educational programmes for physicians, other health professionals and drug regulatory authorities may help in reducing the risks of serious ADRs from NSAIDs and for devising procedures to reduce their risks.

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S20

Development and characterization of an animal model of chronic pelvic pain due to prostate inflammation.

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Prostatitis is often a painful condition, prevalent in 4–14 % of the general population (Mehik *et al.*, 2003). Of these, more than 90 % of the patients suffer from chronic prostatitis/ chronic pelvic pain syndrome (CP/ CPPS), a chronic painful condition classified as category III prostatitis by NIH (Krieger *et al.*, 1999; Nickel, 2003). Category III prostatitis is subclassified into IIIA and IIIB, depending on the presence or absence respectively, of inflammation of the prostate (Nickel, 2003). Chronic pelvic pain, testicular pain, and pain or discomfort during voiding and/ or ejaculation are some of the common symptoms of category III chronic prostatitis (Nickel *et al.*, 2001; Krieger *et al.*, 1999). Despite its high prevalence and lack of optimal treatment, mechanisms of pain development in prostatitis are poorly understood. This is partly due to the lack of a suitable pre-clinical model.

In the current study, our laboratory developed and characterized an animal model of pelvic pain caused by inflammation of the prostate (NIH category IIIA). Briefly, two separate groups of Sprague-Dawley rats were injected with 25 µl of sterile saline (n=6) or a sterile suspension of 3 % carrageenan (n=6) into the ventral right and left lobes of the prostate, under isoflurane anaesthesia. At different time points after surgery (48 h, 72 h and 1 wk), radiant heat (Hargreave's method) or von Frey filaments with gradually increasing bending forces (mechanical stimuli) were applied to the skin between the penis and the scrotum, scrotal skin overlying the testicles, and the ventral tail root. The latency of escape (s) from the radiant heat source was taken as indicator of heat threshold, and the bending force (g) of the filament to which the animal responded by moving from the resting state was taken as the mechanical threshold.

The animals injected with 3 % carrageenan showed a statistically significant reduction in mechanical threshold (mechanical allodynia) in the scrotal skin at 72 h and 1 wk time points compared to the sham animals. However, no reduction in heat threshold (heat hyperalgesia) was observed in the inflamed animals. To confirm that the observed behavior was indeed due to pain, the model was validated using a standard analgesic morphine. Morphine (5 mg/kg., i.p.) significantly reduced the mechanical allodynia observed in this model at 72 h. We hope that this model will help to study the neurobiological mechanisms of chronic pelvic pain due to prostate inflammation.

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S21

Effect of NSAIDs on the hydrophobic phospholipid barrier of the gi tract and the development of the pc-nsaid technology

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Our laboratory has demonstrated that the gastric mucosa of a number of species including humans has a unique hydrophobic surface properties, that makes it non-wettable to luminal acid. This surface hydrophobic characteristic appears to be attributable to the presence of surface-active phospholipids, notably phosphatidylcholine (PC) within and on the luminal surface of the mucus gel layer. We also have evidence that NSAIDs have the ability to rapidly attenuate the stom-

ach's hydrophobic property, which corresponds with a loss in barrier property. This NSAID-induced disruption of the stomach's hydrophobic barrier appears to be attributable to the chemical association of NSAIDs with PC present on gastric mucus lining, due to both hydrophobic and electrostatic interactions between these two classes of amphipathic molecules (Lichtenberger *et al.* 2006; Lichtenberger 2001). Our lab has developed a strategy to protect the upper GI tract from NSAID-induced surface injury by pre-associating NSAIDs with synthetic or soy PC. The resultant PC-NSAIDs have, in turn, been tested in rodent model systems and demonstrated to induce reduced GI lesions and bleeding (*vs* unmodified NSAIDs) while still maintaining the drugs' therapeutic anti-inflammatory/analgesic efficacy (Lichtenberger *et al.*, 1995; Lichtenberger *et al.* 2007). We have also evaluated two of our PC-NSAID formulations, Aspirin-PC and Ibuprofen-PC in pilot clinical trials in healthy volunteers and osteoarthritic (OA) patients which confirmed that they have increased GI-safety and equivalent bioavailability/therapeutic efficacy to the respective unmodified NSAIDs (Anand *et al.* 1999; Lanza *et al.* 2008).

Conclusion: PC-NSAIDs represent a novel class of NSAIDs with improved GI safety and equivalent therapeutic efficacy as demonstrated in both laboratory animals and humans

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S22

Selective and non-selective cyclooxygenase-2 (cox-2) inhibitors in cancer chemoprevention and therapy: safety and efficacy

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Cohesive scientific evidence from molecular, animal and human investigations supports the hypothesis that aberrant induction of COX-2 and up-regulation of the prostaglandin cascade play a significant role in carcinogenesis (Harris, 2007). Over-expression of the normally quiescent COX-2 gene is induced by a plethora of cancer causing agents resulting in heightened biosynthesis of prostaglandins, particularly PGE₂, which in turn drives essential features of carcinogen-

esis (mutagenesis, mitogenesis, angiogenesis, dysfunctional apoptosis, metastasis and immunosuppression). Reciprocally, blockade of the process has strong potential for cancer prevention and therapy. Notably, both non-selective and selective COX-2 inhibitors used on a regular basis reduce the risk of human cancer and precancerous lesions at all anatomic sites thus far investigated (Harris *et al.* 2006, 2007, 2008).

Results confirming that COX-2 blockade is effective for cancer prevention and therapy have been tempered by observations that some selective COX-2 inhibitors (e.g., rofecoxib) may pose a risk to the cardiovascular system. As a consequence, rofecoxib was removed from the world marketplace and black box warnings were placed on celecoxib and other selective COX-2 inhibitors. Nevertheless, meta-analysis of all published randomized clinical trials and observational studies suggests that increased cardiovascular risk is only associated with specific compounds rather than being a class effect of all COX-2 inhibitors.

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S23

Cyclooxygenase inhibition and cancer treatment. A chance missed?

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The observation that colon cancer occurs less frequently than expected in individuals who have been takers of anti-inflammatory drugs was followed by evidence indicating cyclooxygenase type 2 upregulation in colonic adenomata and carcinomata, and that similarly reduced frequencies were to be found in gastric and oesophageal cancer suggesting a common basis (Harris, 2003). A logical extension of thought was to ask if treatment with COX-2 inhibitors might alter the clinical course in diagnosed cancer.

A clinical trial [VICTOR] was therefore set up, with a design requiring the inclusion of 7000 patients with stage II/III colorectal cancer who had had their cancers resected and had completed any required chemo- or radiotherapy before starting treatment with randomly allocated COX-2 inhibitor or placebo (Kerr *et al.*, and VICTOR Study Group, 2007). The trial was terminated early with 2434 patients entered, following a report of a raised risk of cardiovascular events in a trial

of COX-2 inhibition in adenoma prevention, and showed a similarly raised risk of thrombotic cardiovascular events in drug [rofecoxib] takers compared with placebo takers.

Conventionally trials of treatment of major cancers depend in analysis upon comparisons of all-cause mortality in drug takers compared with controls. However, planned power, to detect a difference in survival of some 3–4%, was lost because too few patients were included, with too short a duration of treatment. Worries about potential risks set against the level of benefit must militate against further trials of similar drugs being carried out unless cardiovascular risks can be ameliorated.

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S24

Clinical aspects of health disturbance tendencies in chernobyl nuclear power plant accident clean-up workers from latvia

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More than 6 000 Latvian inhabitants worked to clean-up Chernobyl nuclear power plant (NPP) accident during 1986–1991. The duration of clean-up workers exposure was 1–6 months including external as well as internal radiation. The aim of these studies was to estimate received external radiation doses and analyse changes in clean-up workers health over the observation period 1987–2007.

Methods: For analysis of health disturbances in NPP accident clean-up workers the data of Latvian Chernobyl NPP accident clean-up workers State Register were used (5399 males, mean age 46,20 ± 0,91). Register includes estimated external radiation doses, passport data, questionnaire with 60 questions and clinical examination results. Control group includes 237 males (servicemen, policemen, drivers, firemen), mean age 46.07 ± 0.99.

Results and Discussion: Detailed analysis of external radiation exposure doses was provided in 2008. External radiation exposure was defined for 3093 persons –57.29%. Minimal received dose was 0.1 mSv (milisivert), maximal –500 mSv, mean 128.6 ± 7073 mSv. We have analysed es-

timated external radiation doses depending from period of exposure and age. In the period of iodine (26.04.1986–06.1986) number of persons with estimated external radiation doses was 623; minimal dose received 0.2 mSv, max. 490 mSv, mean 159.29 ± 64.64 mSv; in non-iodine period (07.1986–12.1986.) number of persons with estimated external radiation doses was 1056; min. dose –0.5 mSv, max. 400 mSv, mean 160.81 ± 62.19 mSv; in the period of 1987 till 1990 year persons with estimated external radiation doses were 1409, min. dose 0.1 mSv, max. dose 500 mSv, mean 91.35 ± 60.36 mSv. Estimated external radiation doses depending of age in the moment of the participation in the accident clean-up works was analysed in two groups 20–29 and 30–70 years old persons; 20–29 years old accident clean-up workers have received min. dose 0.2 mSv, max. dose 400 mSv, mean 147.46 ± 63.16 mSv; 30–70 years old persons min. dose 0.1 mSv, max. dose 500 mSv, mean 116.05 mSv.

The monitoring of over 6000 clean-up workers health condition made it possible to obtain unique data on quantitative and qualitative changes in the morbidity structure and health disturbances in these patients. Their morbidity exceeds age- and sex-matched non-exposed population morbidity; there being a trend for progression of this tendency. The number of diseases diagnosed per person was 1.28 in 1986, 1.49 in 1990, 3.24 in 1996, compared with 8.72 in 1999 (p<0.01), 10.22 in 2002 (p<0.001) and 10.88 in 2007. In control group the number of diseases per person was 1.5 in 1996, compared with 2.4 in 2002 (p>0.05). In 60–65 yrs old Latvian males the number of diseases per person was 7.5 in 2002. Even taking into account more frequent examination of clean-up workers, that could be a sign of premature aging in this group.

We have analysed difference of increase of incidence of the diseases per 100 persons during 1996–2001 in the group of clean-up workers and control group. Clean-up workers have more disturbances of following systems compared with control group. Incidence of the diseases of nervous system and organs of sense in the group of clean-up workers were 57.1 ± 2.7, in control group was 5.9 ± 3.3 (p<0.001); incidence of mental disorders in the group of clean-up workers was 61.2 ± 3.0, in the control group this was 5.6 ± 3.4 (p<0.001); thyroid diseases 19.8 ± 2.4 and 5.1 ± 1.6 respectively (p<0.001); respiratory diseases 29.3 ± 2.6 and 9.7 ± 2.4 respectively (p<0.001) and diseases of digestive system 40.9 ± 3.5 and 20.6 ± 2.9 respectively (p<0.001).

Analysis of morbidity in the period from 2002 till 2007 show changes in the structure of diseases. In 2007 the most frequent were in order (as in the previous period) diseases of nervous system, organs of sense and mental disorders, morbidity with diseases of cardiovascular and endocrine system is increasing in comparison with period 1996–2001 but morbidity with respiratory diseases and diseases of digestive system is decreasing.

The oncological morbidity of Chernobyl NPP accident clean-up workers is progressively increasing. During 1998–2004 increase of incidence of oncological diseases of thyroid gland, prostate and stomach but during 2005–2007 increase of incidence of oncological diseases of prostate, stomach and lungs is higher than that among age and sex matched groups of Latvia's population.

Progression of functional disorders of these systems, together with metabolic disorders, hormonal and immune imbalance leads to formation of so called syndrome of “post-radiation neuro-somatic polyopathy”, which was diagnosed in 89% of clean-up workers.

Conclusions: Clean-up workers health disturbance has a strong tendency to polymorbidity. Clean-up workers exposed by low-dose radiation have signs of premature ageing, but this hypothesis requires further investigation.

S25

Intracellular signalling pathways controlling inflammation: potential for pharmacological control of chronic inflammatory disease

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Technological advances in molecular biology have stimulated a paradigm shift away from low molecular weight chemicals to biologics. The high inherent selectivity and affinity of these agents for their targets compared to low molecular weight drugs (LMWDs) has also given more confidence for enhanced efficacy and safety. This has resulted in a substantial reduction in investment LMWDs. In 2007, only 13 of 492 trials involved new chemical entities (www.clinicaltrials.gov). Biologic medicines have dramatically widened our knowledge of disease mechanisms through successes, such as TNF α and IL-6 therapy in rheumatoid arthritis, but also failures, such as α IL5 in asthma, and α CCL2 in arthritis. The success of the former targets and the failure of the latter are thought to reflect the hierarchical level of the target, TNF α having a being near the peak, and CCL2 being at the effector level, prone to redundancy. LMWDs have the advantage of being available intracellularly, and offer titratability. Intracellular targets for LMWDs offer alternatives to biologics with an intracellular hierarchy. LMWDs.

Current intracellular LMWD targets being investigated in the clinic include JAKs, MAPKinases, and PDE-IV. Others in preclinical investigation include steroid receptors, IKK and PI3kinase. Notable in these series is the fact that the majority are kinases, and the dissociated steroids modulating kinases. Investigations into p38 MAPKinase are the most mature (Schett *et al.*, 2008). Trials have raised safety issues related to p450, which have now been designed out. However, the last remaining trial was withdrawn late 2007 for low efficacy, biologics having raised the stakes as regards ACR responsiveness. The EU funded MACROCEPT and KINACEPT projects have aimed to improve both cellular selectivity of p38 inhibitors and efficacy through drug targeting to myeloid cells. We have found that certain p38 inhibitors

possess prolonged pharmacodynamic activity selective for dendritic and accessory cells over lymphocytes (Moradi *et al.*, 2008). This may be due to downstream kinases producing certain phosphorylation patterns. In addition, myeloid targeting enhances disease modifying activity in the dextran sulphate colitis and collagen arthritis in mice (Guse *et al.*, 2007). Increasing the efficacy of p38 inhibitors could also be achieved using the new “promiscuous” drugs, that inhibit two or more diverse targets, one pair being p38 and PDE-IV, or even three including adenosine receptor antagonism (Trifilieff *et al.*, 2005). The inhibition of JAK3 is looking promising, with results promised for the American College of Rheumatology meeting this year. JAK3 as a target confers some selectivity, its deficiency being associated with the haematopoietic system to create a form of SCID. This raises the obvious question as to the level of suppression required for JAK3 suppression to be disease modifying, without conferring profound immunodeficiency. Here is the advantage of the titratability of LMWDs.

Whilst NF κ B has been a core research subject since its discovery, investigations have attempted to increase selectivity of action and included the IKKs that control its activity. Whilst research has concentrated on IKK, we have unexpectedly found that IKK α plays a role in T cell responsiveness to antigen presentation (Johnson *et al.*, 2007), as opposed to non-specific ConA induced responses⁴. Radiation-reconstitution chimaera experiments are beginning to support this conclusion. IKK α is usually considered to induce an aggressive inflammatory signal. Thus, this molecular may act as a pivot between the innate and adaptive system. The advantage of this is that its suppression in auto-immune disease may leave the innate system intact. LMWDs acting on intracellular targets provide hope in filling the gaps left by biologics.

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S26

Soluble mediators and cellular players in acute inflammatory resolution

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Inflammation is a primordial response to infection and injury that seeks to neutralise and eliminate foreign organisms and/or material. Thus, inflammation is no trivial event. Life depends upon it. In general, the innate inflammatory response initiates within minutes and resolves within hours. Chronic inflammation, on the other hand, persists for weeks, months or even years and, unlike the acute response, is the side of host immunity we need to avoid. Notwithstanding, dispersed among this black and white view of inflammation are shades of grey. We are constantly reminded that defining inflammation is not so easy and that while acute inflammation can resolve, it can also be recurrent and that, over time, chronic inflammation can also resolve or persist with devastating consequences to the host.

In this presentation, one of these many aspects of the inflammatory response – how acute inflammation resolves, will be discussed. In doing so it will be argued that resolution is an active process, whose failure may predispose the host to chronic inflammatory diseases and autoimmunity. At the very least it is hoped we can highlight resolution as a critical facet of the inflammatory response and serve to underline the importance of not altering its normal course of action when developing novel anti-inflammatory drugs. Ultimately, it will be proposed here that resolution is controlled by endogenous pro-resolution factors, which, for the future, may represent a treasure trove for drug discovery in terms of designing drugs that mimic their mode of action or enhance their synthesis (Serhan *et al.*, 2007). Specifically, earlier work will be reviewed that was done on cyclooxygenase and lipoxygenase-derived lipid mediators (cyclopentenone prostaglandins, prostaglandin D₂, lipoxins and aspirin-triggered epi-lipoxins) (Gilroy *et al.*, 2004; Lawrence *et al.*, 2002) in limiting the continuity of the inflammatory response; bring the audience up to date with a more recent understanding of some of the cellular players in this setting and introduce some new soluble mediators (resolvins) and their receptors as potentially novel pro-resolving agents. In this setting, macrophage phenotype as a critical determinant of inflammation longevity will also be discussed.

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S27

Electronic data capture (EDC) using cellular technology: implications for clinical trials and practice

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Aim: The capture, analysis and utilisation of health status information are attended by logistic considerations and interpretation challenges. Electronic data capture (EDC) can provide a method that is valid, reliable, easy, fast and convenient. Previously, EDC has mainly relied on desktop, laptop or handheld computer devices. Most recently, advances in cellular technology have provided opportunity to collect health status information using mobile phones. In order to explore this issue in OA we have conducted a preliminary evaluation of cellular technology in capturing WOMAC[®] NRS 3.1 Index data using three different mobile phone brands, in order to select one phone for a future validation study of m-WOMAC[®] e-capture.

Methods: A Java-based application of the WOMAC[®] NRS3.1 Index was developed by Exco InTouch for delivery by mobile phone (m-WOMAC[®]). Currently available mobile phones were reviewed and one phone identified from each of three major international brands (Nokia 6300, Motorola V3, Samsung A711). Suitability of phones was based primarily on the size of the phone, keypad, navigation pad and screen. WOMAC[®] NRS3.1 application functionality was reviewed by a rheumatologist and physiotherapist. Feedback to Exco InTouch, informed further optimisation. Twelve patients with symptomatic osteoarthritis (OA) of the hip and knee were enrolled in the pilot study. Following task orientation, patients completed the three m-WOMAC[®] applications in random-order.

Results: Patients (2 men and 10 women) with hip (n=4) and knee (n=8) OA participated (mean age=67.2 years, range=55–82 years, mean disease duration=8.4 years). Ten patients had concomitant hand OA.

All patients successfully completed the m-WOMAC[®] Index on each of the three phones, and all were found acceptable. Using Friedman's ANOVA no significant difference between the 3 phones with respect to m-WOMAC[™] mean overall rank score was found (ANOVA chi squared=2.2, p=0.34). However, Motorola v3 was favoured with the highest mean rank 1.75, SD=0.75, followed by Samsung 1.91, SD=0.90. It was noted that 8 patients used the keypad and 4 used the navigation pad, to enter their scores

While not the prime focus of this study, Pearson correlations were calculated to examine the strength of association between p-WOMAC[®] and m-WOMAC[®] scores. The Pearson correlation between the average p-WOMAC and the patient's corresponding average m-WOMAC[®] score was 0.996. There was no statistically significant difference (paired t-test) between the average p-WOMAC[®] (4.05) and m-WOMAC[®] (4.11) scores (p=0.43)

Conclusions: Twelve patients with hip and knee OA, (10 with concomitant hand OA), successfully completed EDC by cellular technology using the m-WOMAC[®] NRS3.1 Index. Patient reported ratings indicated the application performed well on all three phones. In comparative ratings, the Motorola v3 was slightly more favoured than the Samsung or Nokia. Further validation of the m-WOMAC[®] NRS3.1 Index is planned. EDC provides unique opportunities for using quantitative measurement in clinical trials and practice.

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S28

Gastric Adaptation to Aspirin administration: Physiological mechanisms and clinical impact

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Lipoxygenase (LOX) products lipoxins (LX) and recently identified COX-2 derived “aspirin-triggered lipoxin LXA₄” (ATL) are unique lipid mediators with a potent anti-inflammatory properties (Wallace *et al.*, 2005). ATL enhance the

resistance of the gastric mucosa to aspirin (ASA)-induced gastric damage (Fiorucci *et al.*, 2002; Souza *et al.*, 2003; Wallace, 2006), but their role in the mechanism of gastric adaptation to ASA has been little studied.

We studied the effect LXA₄ administration against the formation of ASA-induced gastric damage and the involvement of ALT and NO in gastric adaptation to acidified ASA (100 mg/kg-d) given p.o. for 5 days without or with concurrent daily treatment with (1) NOS inhibitor, L-NNA, (2) selective COX-2 inhibitor (rofecoxib) and (3) 5-LOX inhibitors, baicalein and A-861. The area of gastric lesions was determined by planimetry. Gastric blood flow (GBF) was assessed by H₂-gas clearance method. Mucosal ALT levels were measured by ELISA and malondialdehyde (MDA) concentrations as an index of lipid peroxidation were assessed in the gastric mucosa.

Single exposure to ASA produced gastric lesions and decreased GBF while increasing mucosal ATL and MDA concentrations. LXA₄ (0.1-20 µg/kg i.g.) dose-dependently reduced the area of ASA lesions and significantly raised the GBF. After 5 repeated exposures to ASA, the lesion area was reduced by 85 % from that at day 0 and an increase in the GBF and a further rise in mucosal ATL levels were observed and these effects were significantly attenuated by L-NNA, rofecoxib, baicalein and A-861. Strong signals for COX-2-, cNOS- and iNOS mRNAs expression were observed in the ASA-adapted gastric mucosa.

We conclude that COX-2 derived ATL induced by ASA are essential mediators of ASA-induced gastric adaptation acting due to activation of NO-NOS system and inhibition of lipid peroxidation.

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P1

Women With Pain due to osteoarthritis: Efficacy and Safety of a once-daily formulation of Tramadol

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Women with osteoarthritis (OA) have a greater severity of disease compared to men, and are more likely to be symptomatic for the same degree of pathologic severity (Srikanth *et al* 2005). Tramadol (an atypical opioid) is a useful therapeutic option for the relief of pain since it, unlike NSAIDs and COX-2 inhibitors, does not produce gastrointestinal bleeding nor has the potential for renal or cardiac adverse events (Kean *et al.* 2008).

This analysis assesses the efficacy and safety of treatment with a once-daily oral formulation of tramadol (Lenaerts *V et al.* 1998) for up to 12 weeks, compared with placebo in women with moderate-to-severe pain due to osteoarthritis of the knee.

Two parallel, placebo-controlled phase 3 clinical trials were analyzed; patients were randomized to a fixed dosage of Tramadol Contramid[®] OAD 100, 200, or 300 mg daily, or placebo. The primary efficacy endpoints were the percentage difference from baseline of the Western Ontario and McMaster Universities (WOMAC) osteoarthritis index subscale scores for pain and physical function and the patient global rating of pain relief after 12 weeks of maintenance therapy. The analysis included 405 women receiving tramadol and 280 receiving placebo.

At week 12, 179/204 (87.7%) women receiving tramadol rated their overall pain relief as effective or very effective, compared with 134/177 (75.7%) receiving placebo. The percentage improvements from baseline of WOMAC pain scores were significantly better than placebo for the 100 mg (58.8% ± 37.1%, $p=0.018$) and 300 mg (58.9% ± 38.8%, $p=0.023$) treatment arms; however, the 200 mg dosage lost significance (53.0% ± 38.5%, $p=0.175$). The WOMAC physical function scores showed significant improvement for the 100 mg (56.9% ± 36.4%, $p=0.009$), 200 mg (54.0% ± 33.8%, $p=0.034$), and 300 mg (53.4% ± 41.4%, $p=0.043$) daily dosages. A time-weighted analysis revealed highly statistically significant improvements over placebo for all WOMAC subscale scores across all dosages.

In conclusion: for moderate-to-severe pain due to OA of the knee, women experience significant analgesia and improvement of physical function over time with treatment with Tramadol Contramid[®] OAD.

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P2

Safety and efficacy of intramuscular sodium ribonucleinate [Osteochondrin[®] S] for relief of pain and joint functions in knee osteoarthritis

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Osteochondrin[®] S [OST] is a natural ribonucleotide extract of bovine-derived connective tissues (from accredited BSE-free sources) and yeast which has been found to reduce joint damage in some animal models of joint injury (Rainsford, 1996) and inhibits cytokine-induced cartilage-bone degradation *in vitro* (Rainsford *et al.*, 2008). Symptom trials have been performed showing that this drug is effective in controlling indices (WOMAC) of joint pain and physical function in osteoarthritis [OA] (Rainsford, 1996; Rainsford *et al.*, 2004). However, a comprehensive clinical evaluation of the safety in relationship to the effectiveness of this preparation in patients with OA has not been performed previously. Hence, in the present study, we undertook a randomised, placebo-controlled, parallel-group study in 166 patients (of whom 145 were evaluable) with OA of the knee at 20 centres in Germany performed under ICH-GCP conditions, the design of this study and part of the efficacy determinations have been reported previously (Rainsford *et al.*, 2004). We also report results of observations on the safety of OST from investigations from a related study in Moscow and previous clinical trials and spontaneous reports.

Materials & Methods: OST (prepared according to GLP and GMP) was used for 4 weeks in a dosage of 3 x 2 ampoules per week. Each ampoule contained 6.3 mg sodium ribonucleinate in 5 mL isotonic medium containing amino acids. The placebo treatment [PLA] comprised 5 mL ampoules of isotonic medium which contained amino acids. In the first series i.m. injections of 20 ampoules were followed by a 9–10 week follow-up period. The trial was performed for a total of 3 periods based on 2 or 3 series of injections and 3 follow-up periods. The primary measure was the O'Brien rank sum of changes from baseline of pain and joint stiffness in the WOMAC index and the intake of ibuprofen as a rescue analgesic after each 3 month period.

Adverse events were recorded at each treatment visit and categorized according the WHO specific terms. Blood samples were drawn at each visit and subjected to full clinical chemistry and haematological screening.

Results:

(1). Safety – Adverse Events (AE) Recorded:

In total 111 events were observed in 48 cases (57.1%) in the OST group *c.f.* 142 in 53 cases (64.6%) in the PLA group.

The relationship to the study treatment was assumed to be related to treatment in 5 cases (6.0%) in the OST group and 6 cases (7.3%) in the PLA group. Four cases each in the OST and PLA groups were deemed serious. No statistically significant differences occurred between the two groups in the incidence of adverse events and in the type of adverse event as determined by their System Organ Classification (SOC). With the exception of one case of arthralgia in the PLA group the others were minor symptomatic reactions unrelated to treatments. Of the 3 serious cases of AEs in the OST group it was determined that there was no relationship to the drug. In another the relationship to OST was considered unlikely. In two other cases the relationship to OST though considered non-serious resulted in their withdrawal from treatment. There were no deaths or serious sequelae.

Laboratory investigations did not reveal any serious abnormal values in OST or PLA groups from respective baseline values. Leucocyte and other changes were small and no of any major clinical significance. There were no changes in eosinophil counts that might reflect any underlying allergic reactions.

(2). Efficacy – Effects on the WOMAC Index Scales: The data obtained in ITT analyses of valid cases can be summarised:

1. Although a relatively high placebo reactor rate was observed there was a statistically-significant reduction in pain scores and overall WOMAC index scores.
2. Using the criteria for the classification of “responders” from “non-responders” in which there was high relief of pain as evidenced by reduction in WOMAC scores > 150, there was a significant reduction in all WOMAC parameters (pain, stiffness and physical function) as well as the overall WOMAC index scales. The percent responders in the high pain relief group was 45.8% for OST and 38.4% for PLA. In those patients where there was moderate improvement in pain (defined as improvements in WOMAC scales for pain relief of 50–149 and function scores of > 340 with patients’ global assessment at least moderate) the percentage responders was 36.1% for OST and 31.5% for PLA.

(3). Other safety observations: Additional investigations were performed (using a similar protocol to that in the German multi-centre study) in 48 patients with OA under the auspices of Prof. Dr. R.M. Balabanova at the Institute of Rheumatology, Moscow. In the safety evaluation there were no recorded cases of serious or non-serious reactions with OST and only one non-serious case from PLA.

In 35 years up to 2000 (i.e. before the initiation of the multi-centre study in Germany and in Moscow) in which OST has been used clinically, there have been 25 cases of adverse reactions reported arising from use of OST or related products. These comprise 40 reports of symptoms of adverse reactions and, additionally, 18 were reported in a clinical trial. Local reactions at the injection site were the most frequent. There were no reports of severe or serious reactions.

Conclusions: (1) OST has a safety profile that is comparable with that of PLA. The incidence and severity of AEs from treatment with OST in clinical trials is low and notably less

than that seen in many trials with NSAIDs or disease-modifying agents used in OA (e.g. glucosamine, hyaluronic acid). (2) OST showed superior efficacy over PLA but because of high placebo reactor rates this was more pronounced when the evaluation was performed using responder criteria.

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P3

Autoimmune diseases revisited

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With all the great advances of the last century, the autoimmune diseases seem to stand out as a group, by the lack of progress in understanding their etiology and prevention. I believe the points below partially refute the idea of a single, universal, self-generated, allergic autoimmune reaction theory.

The distribution of joint involvement in Rheumatoid Arthritis (RA) (Dieppe *et al.*, 1985), an accepted autoimmune disease, is extremely unlikely to be due to an autoimmune attack on cartilage. With the great majority of cartilage being of the same constituency why should the distribution be so specific and symmetrical? Symmetry and typical anatomical distribution (Dieppe *et al.*, 1985) directs one’s thoughts to a neurogenic involvement. Patients who suffer a CVA before or after the onset of RA are noted to have amelioration of joint involvement in the affected limb. Nerve injuries protect the area from the onset of RA. Interestingly, the same occurs with Heberden’s nodes, significant resolution follows nerve injury.

Joints prone to RA are those with increased substance P afferent nerve endings (Payan, 1992). Antidromic stimulation causes the release of substance P and inflammatory joint changes.

The administration of capsaicin to neonatal rats considerably reduced the effect of induced experimental arthropathy (Lam and Ferrell, 1989; Lorton *et al.*, 2000; Warsame Afrah *et al.*, 2004) and in rheumatoid arthritis (Matucci Cerinic *et al.*, 1995). Involvement of certain tendons such as the Achilles in cases of R.A collates well with the high substance P nerve fiber innervation.

Numerous case reports at the beginning of the last century reported significant improvement in cases of arthritis,

particularly the inflammatory form, following ganglionectomies.

In the 1960's it was noted that patients on phenytoin for epilepsy had a reduced instance of R.A. and this was followed by a number of small double blind studies demonstrating that phenytoin was effective on the clinical and biochemical manifestations (Grindulis *et al.* 1986; Rao *et al.*, 1995). Modern trials, in developing countries that can't afford modern rheumatoid disease modifying drugs, show very positive results (Rao *et al.*, 1995) Interestingly, today pregabalin and gabapentin are being evaluated in the management of arthritis.

In many case of Juvenile Arthritis with inflamed joints and toxic symptoms local intra-articular injections of cortisone produce a very rapid and significant improvement, albeit short lived in weeks, in the general condition. This suggests that it is the joint that is the center of the stimulus to the toxicity and blood changes. In our own experience with adults marked improvements lasting weeks if not months have followed intra-articular local anaesthetic agents without the addition of cortisone. Could this be breaking a neurogenic cycle by blocking nerve endings? Similarly the positive effects of injections of local anesthetics in treating trigger points and myofascial pain may have the same underlying mechanism of effect, with very positive staining for substance P (Scudds *et al.*, 1995).

Most cases of Vitiligo (Laberge *et al.*, 2005) that I have seen, have been very symmetrical in de-pigmentation, it is nigh impossible to imagine a localized allergic reaction to specific melanocytes or such localized areas. Often an area of depigmentation is preceded by paraesthesia. After depigmentation the skin becomes insensitive to capsaicin.

In a similar vane fibromyalgia, with its symmetrical distribution of tender points and abnormal substance P concentrations, possibly suggests a muscular form of the above joint patterns and aetiology (Lucas *et al.*, 2006).

Extensive work on peripheral nerve section prior to induced arthropathy in animals, again demonstrates the very marked involvement of the neurogenic input rather than the allergic reaction (Matucci Cerinic *et al.*, 1995). Most surprising, the discovery that modification of pancreatic sensory nerves can effectively alter islet inflammation and insulin resistance (Razavi *et al.*, 2006).

The present focus of autoimmune disorders is that in dealing with the cascade inflammatory effect, little attention is paid to the neurogenic involvement and its cause. The retrograde passage of substance P in neurons, *i.e.* two-way traffic, is very atypical and must have a reason.

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P4

Effects of ibuprofen and anti-oxidants on biochemical and Clinical aspects of health status of Chernobyl clean-up workers from Latvia

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Following the Chernobyl nuclear reactor explosion in April 1986 about 6000 male workers from Latvia were involved in the clean-up of the reactor site during the period of 1986–1990. External radiation exposure was estimated for about 40% of clean-up workers from Latvia. The recorded highest doses they received did not exceed 200 mSv. However, most of workers were exposed to large quantities of toxic radio-active isotopes and heavy metals released from the melt-down of the reactor. Our previous biochemical investigations (Kumerova *et al.*, 2000) provide evidence that these workers have sustained oxidant stress injury. Thus, in the current study we have investigated the possibility that intake of anti-oxidants may confer some benefit on these patients. Moreover, since non-steroidal anti-inflammatory drugs such as ibuprofen can control the inflammatory reactions leading due to tissue injury by oxidants (Ward *et al.*, 1995) and is amongst the safest of the NSAIDs ibuprofen (Rainsford, 1999) may in combination with anti-oxidants exert protective effects against oxidant-stress injury in the Chernobyl clean-up workers.

Aims: In order to establish if anti-oxidants and anti-inflammatory drugs have some protective effects on the manifestations of oxidant stress injury we investigated the effects of long-term treatment with either selenium + vitamin E or low dose of ibuprofen on plasma levels of oxidants and anti-oxidants and quality of life parameters in a clinical trial of patients recruited from the Latvian Registry of Chernobyl Clean-up Workers.

Methods: This was randomized, double-blind, placebo-controlled, parallel group investigation involving 82 male Chernobyl clean-up workers, who participated in clean-up works after Chernobyl nuclear power plant disaster from 1986–1989. At the time of participation in the study in 2005 their ages were 38–64 years (49.96 ± 5.60 , mean + s.d.). Of those recruited 51 had received radiation doses of 135.72 ± 68.81 , mean + s.d.) mSv. All participants were randomly divided into the following treatment groups: (1)

group A (N=21) received vitamin E 350mg/d and selenium methionine 200µg/d; (2) group B (N=20) received ibuprofen 400mg/d; (3) group C (N=21) received vitamin E 350mg/d, selenium methionine 200µg/d and ibuprofen 400mg/d; and (4) group D (N=20) received placebos of identical appearance to both the anti-oxidants and ibuprofen. The treatments lasted for 12 months. Clinical observations and biochemical analyses including plasma levels of vitamin E, Se and prostate specific antigen (PSA) measured by immunoassay as well as routine clinical chemistries. A standard Quality of Life (QoL) questionnaire (Nosikov & Gudox, 2003) was employed to determine the health status of the patients; the questionnaires were completed by one of the clinicians upon interview of the subjects and were conducted at 4 periods during the trial *i.e.* at the first visit, then at 3, 6 and 12 months after the start of the trial. Additionally, follow-up interviews were conducted in groups C and D one year after treatment discontinuation. The questionnaire included 12 questions about person's subjective opinion on his health condition, his quality of life and the intake of other medicines.

Results. At the beginning of trial nearly 50% of all patients suffered from selenium insufficiency. Thus, 13 subjects (15.9%) from 82 which were included had severe Se deficiency (plasma levels $53.15 \pm 1.56 \mu\text{g/L}$; mean \pm s.d.) while a further 26 (31.7%) had clinically significant Se insufficiency ($73.1 \pm 1.0 \mu\text{g/L}$ mean \pm s.d.). Plasma levels of vitamin E in all groups in the beginning of the trial were about half of normal values. These ranged in average values from 9.65 mg/L in Group C (Se + vitamin E + ibuprofen) to 11.4 mg/L in Group B (ibuprofen). After 3 and 6 months period there was a significant increase in levels vitamin E accumulation in 24.3% of patients while after 9 and 12 months the plasma levels were $23.13 \pm 1.49 \text{ mg/L}$ and $24.96 \pm 1.60 \text{ mg/L}$ (mean \pm s.d.), respectively, in groups A and C. There were 60 analyses where the vitamin E levels exceeded normal values in these groups that received vitamin E. Aside from these individual group changes, there were no statistically significant differences observed overall between the groups.

In the QoL assessments almost in all groups some improvement was observed initially, but a decline in positive parameters was evident after 6 months period, and after 1 year period the subjective evaluation of respondents' health status was the same as in the beginning of the study. These changes may reflect seasonal changes in mood and perception. Aside from these changes, the QoL in group C did not reflect deterioration even a year after the study.

The clinical chemistry data showed that there were a few patients in each of the groups had high liver enzymes and bilirubin but overall there were no differences between the treatment groups and no indications that there were abnormal levels in particular treatment groups. Likewise, aside from a few patients with abnormal levels in serum urea, creatinine or TSH levels there were no overall changes associated with any of the treatments. Most patients had normal or near normal PSA levels, but two in group D and one in group B had radical prostatectomies during the period of treatments.

During this study 7 new cases of cancer were discovered among the research participants, one patient was operated

due to cancer before trial beginning. Patients were operated due to prostate, stomach and intestine (appendix) malignant tumors.

Conclusions. The results show that intake of Se + vitamin E (with or without ibuprofen) for more than 6 months does not lead to any increase in plasma levels of anti-oxidants. This may reflect endogenous metabolism of anti-oxidants as a consequence of the oxidant-driven inflammatory diseases or conditions of the patients. After 6 months plasma vitamin E concentrations show a trend to exceed normal values but it is not known if this is clinically significant.

The most notable improvements in QoL as well as the subjective evaluations of health are apparent between 3rd and 6th month from initiation of the trial. After the 6 months there is a decline in positive outlook of patients. This may be a consequence of seasonal or personal factors.

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P5

Effects of antioxidant mixtures on biochemical (oxidant/antioxidant) status and clinical observations in Chernobyl clean-up workers from Latvia

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Most of the Chernobyl Nuclear Power Plant (NPP) disaster clean-up workers from Latvia have progressive multiple illnesses that exhibit tendency to progress; their morbidity exceeds that observed in general Latvian male population. Most of these workers have up to 11 and more different illnesses. According to data obtained during the thorough examination (1990–2007) of the Chernobyl clean-up workers from Latvia, great part of these people has progressive dis-

turbances in functioning of immune system and increasing morbidity with oncological diseases (Zvagule *et al.*, 2002) with evidence of prolonged systemic oxidant stress injury (Kumerova *et al.*, 2000).

The Aim of this Study was to determine the effects of prolonged therapy with antioxidants on the oxidant/antioxidant status and health condition of Chernobyl NPP clean-up workers from Latvia.

Materials and Methods: A randomized, parallel group investigation was performed over a one year period in 2007–2008. A total of 52 patients were examined in course of this study. They were males, who participated in clean-up works after Chernobyl nuclear power plant disaster from 1986 until 1989. At the moment of participation in clean-up works their age was between 19 and 45 (mean value of age 30.46 ± 6.06). Their age in 2007 was 40 to 66 years (mean value of age 50.98 ± 5.97). All participants were divided into three groups in random order: group A – 10 males, who received vitamin E 350 mg/d and selenium methionine 200 µg/d; group B – 16 males, who received vitamin E 350 mg/d, selenium methionine 200 µg/d and coenzyme Q₁₀ 100 mg/d; and group C – 26 males, who received selenium methionine 200 µg/d alone for 12 months. They attended the clinic 4 times (before supplementation, after 3, 9 and 12 months) for detailed clinical examinations and collection of plasma for some biochemical analyses (levels of vitamin E, selenium)

Results for:

A group (Se+Vit E)

Se 78.3 µg/L (before suppl.) – 111.3 µg/L (after 3 months suppl.) 131.0 µg/L (9 months) and 126.4 µg/L (12 months); Vit E (accordingly) 12.2 mg/L – 18.78 mg/L – 15.18 mg/L and 18.04 mg/L

B group (Se+E+Q₁₀)

Se 82.1 µg/L – 110.3 µg/L – 142.5 µg/L – 128.3 µg/L
Vit E 17.66 mg/L – 19.65 mg/L – 16.5 mg/L and 23.83 mg/L

Group C (Se)

Se 79.7 µg/L – 126.8 µg/L – 148.0 µg/L and 137.1 µg/L
Vit E 14.29 mg/L – 13.57 mg/L – 14.27 mg/L and 17.96 mg/L.

A Quality of Life questionnaire (Stark D. *et al.* 2002; Nosikov and Gudex, 2003) was applied over these 4 three-monthly periods, *i.e.* on the first visit, then at 3, 6 and 12 months after initiation of the trial. The questionnaire included 12 questions about person's subjective opinion on his health condition, his quality of life and the concomitant intake of medicines. The results of study were statistically processed using programs Microsoft Excel and SPSS Data Editor 11.0 version.

Results: There were no significant differences in the quality of life parameters between the treatment groups with the exception of those in group C in relation to ability to perform daily activities. There was an increase in the number of patients with positive answers in the category of "good" or "moderate" ability to perform daily activities, which were 72% on the first visit vs. 80.9% after 1 year. In contrast there was deterioration in these categories in groups A and B. After 1 year of treatment respondents in group B and C were more satisfied with their health status than in the beginning of the

study and compared with group A. Concerning other questions, almost in all groups initial improvement was observed, but unfortunately the decline in positive results was noticed after 6 months period, and after 1 year period the subjective evaluation of respondents' health status was the same as in the beginning of the study. Biochemical analysis of group A after 3 months period significant vitamin E accumulation has showed in 45.5% of cases.

In 36 analyses (in all three groups) content of Vit E were more than 20 mg/L (normal ratio 5–16 mg/L), max. 40,4 mg/L. Number of all Vit E analyses – 181, with Vit E overload – 36 (approx. 20%).

In Conclusion this study shows, that intensive accumulation of vitamin E is limiting factor for prolonged usage of it as antioxidant. Intensive accumulation of vitamin E reaches toxic limits in blood in short time period and early may cause toxic effects. Supplementation with vitamin E in conventional therapy after vitamin E laboratory examination may be recommended for long-term usage, only regularly controlling levels of vitamin E in blood. Long-term treatment with selenium and coenzyme Q10 for more than 6 months may be effective in improving ability to perform daily activities and general quality of life as subjective evaluation of health status, how it was shown in results of inquiry. Initial improvement in subjective evaluation of quality of life and health condition in respondents with following worsening probably may be associated with psychological effects of treatment, which need closer investigation.

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P6

Gastrointestinal (GI) and cardiovascular (CV) risks associated with the use of anti-inflammatory drugs are influenced by Th1/Th2 dichotomy: a novel concept

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Th1-predominant, C57BL/6 mice are more resistant to NSAID-induced gastric and duodenal damage than Balb/c mice suggesting an increased GI risk in Th2 individuals taking NSAIDs (Padol et al., 2001). However, the opposite is true for CV risk since Th2- predominant, Balb/c mice are more resistant to developing atherosclerosis while C57BL/6 are more prone (Paigen B et al., 1985).

We have shown that in Th1-predominant C57BL/6 mice, which interestingly lack sPLA₂, PGE₂ does not inhibit acid secretion because these mice express ~5 times less EP₃ receptors in gastric mucosa compared to Th2-predominant Balb/c mice that show intact PGE₂ physiological antisecretory responses (Padol & Hunt, 2005). These observations may determine a lower GI risk associated with NSAIDs use in Th1-predominant individuals.

Atherosclerosis is a Th1-driven disease and unstable atheromatous plaques are characterized by inflammatory cells, particularly T-cells, neutrophils and macrophages, which contain lipids, predominantly LDL, leading to foam cells. In contrast, stable plaque is associated with a Th2 immune response and is predominantly composed of smooth muscle cells, collagen secreting cells and collagen. Statins influence a shift from a Th1 driven to a Th2 driven inflammation (Ghittoni R et al., 2007), resulting in attenuation of atherosclerosis and remodeling of atheromatous plaque. This favorable shift from the inflammatory cell-based, toward the more organized and stable smooth muscle and collagen composed plaques, results in a lower risk of CV events such as myocardial infarction (MI). Prostaglandin(s) express an immunomodulatory role and skew the immune response toward Th2 (Shibata Y et al., 2005). Consequently, prostaglandin(s) inhibition by anti-inflammatory drugs results in a reversal of this shift and augmentation of an inflammatory Th1 response (Harizi H et al., 2002). Inhibition of COX-2 by both selective and tNSAIDs is associated with an increased incidence of MI. Therefore, we postulate that chronic use of COX-2 selective and tNSAIDs and consequent prostaglandin inhibition results in immunomodulation toward a Th1 response that exacerbates atherosclerosis, leading to unstable plaque, which subsequently increases the incidence of MI. This novel concept emphasizes the chronic immunomodulatory effect of anti-inflammatory drugs, which results in a CV risk associated with augmentation of the Th1 response. In contrast, GI risk associated with NSAIDs use is acute and physiological in nature.

Thus, GI and CV risk with the use of anti-inflammatory drugs are influenced by the Th1/Th2 dichotomy and mutually exclusive. Our new concept should lead to better patient stratification and better drug and therapy design.

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P7

How safe is paracetamol as an alternative analgesic to NSAIDs?

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Background: Paracetamol is the favoured analgesic for osteo- and rheumatoid- arthritis, being generally considered non-gastrotoxic and not causing cardiovascular complications – in contrast to NSAIDs. Clinical evidence to support this view is questionable (Garcia-Rodriguez and Hernandez-Diaz, 2001a,b).

Aims: (i) To establish if paracetamol is without gastric irritation in rats exposed to inflammatory conditions and hyperacidity as experienced by many patients with rheumatic disease; and (ii) To determine if paracetamol affects beneficial immune responses in a rat model of arthritic disease.

Experimental: (i) The gastrotoxicity of oral paracetamol was examined in rats with various degrees of inflammation with/without co-administration of isotonic hydrochloric acid. (ii) Induction of tolerance, to prevent collagen-induced arthritis in rats, by Peptacan (Ghosh et al., 2006) was studied in rats administered paracetamol at analgesic/antipyretic doses (150 mg/kg).

Results: Paracetamol mimicked aspirin causing significant gastric damage when animals suffered inflammatory stress and there was acid in the stomach (pH ≤ 3). Paracetamol also negated the prevention of collagen-induced arthritis by oral Peptacan, a natural tolerogen produced during cartilage breakdown in OA.

Conclusions: These studies highlight potentially contentious issues about the GI and immunological safety of paracetamol when used to treat patients with rheumatic diseases, especially if there are profound inflammatory reactions and patients are hyperacidic. More damaging in the long term may be the tolerance-destroying effect of paracetamol, preventing natural auto-regulation of inflammatory cartilage attrition by cartilage fragments.

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P8

Intestinal permeability and inflammation in rats given indomethacin and antibiotics

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Background and Aims: Nonselective COX inhibitors like indomethacin increase intestinal permeability and cause intestinal inflammation and ulcers in rats (Somasundaram *et al.*, 2000; Sigthorsson *et al.*, 2002; Hotz-Behofsits *et al.*, 2003). Germ free animals or animals treated with antibiotics show little or no macroscopic damage of the gastrointestinal tract following ingestion of conventional NSAIDs. Uejima *et al.*, 1996. We investigated whether intestinal permeability and intestinal inflammatory changes are equally influenced by antibiotics or whether permeability and inflammation are affected to a different degree.

Methods: Intestinal permeability ($^{51}\text{CrEDTA}$) and inflammation (faecal granulocyte marker protein (GMP)) were assessed using methods described (Sigthorsson *et al.*, 2002; Hotz-Behofsits *et al.*, 2003) at baseline and after dosing with 100 mg/kg bodyweight amoxicillin & 50 mg/kg bodyweight clavulanic acid and/or 10 mg/kg bodyweight indomethacin.

Results: Indomethacin increased intestinal permeability and caused intestinal inflammation. Pre-treatment with amoxicillin/clavulanic acid reduces the permeability increase and abolishes the inflammation. The Pearson correlation was used to test for correlation between permeability and inflammation. The correlation between permeability and inflammation after a single dose of NSAIDs in rats was 0.85 (Pearson correlation), $p=0.001$.

Conclusions: The different reaction of intestinal permeability and GMP to antibiotics treatment is consistent with the suggestion that permeability changes precede any inflammation in the GI tract. It is also apparent that antibiotics reduce the permeability changes. Hence the idea that NSAIDs increase intestinal permeability by a topical action on mitochondrial oxidative phosphorylation is supported, but the magnitude of permeability change is further dependent on luminal aggressors (bacteria). Therefore the permeability and inflammatory changes are inter-dependent.

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P9

Methylamine increases nitric oxide synthase activity of rat white adipocytes: a new role for the semicarbazide-sensitive amine oxidase?

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Background: Methylamine (MET) is the shortest ammonia (NH_3) derivative circulating in mammals from degradation of endogenous or diet-deriving amines. (Zeisel & daCosta, 1986). Notwithstanding tissue levels of MET are found increased in inflammatory-based pathologies as diabetes and Alzheimer's disease (Ferrer *et al.*, 2002) their physio-pathological significance is unclear yet. MET is scavenged by the activity of a semicarbazide-sensitive amine oxidase (Bz-SSAO) which oxidatively deaminates MET producing hydrogen peroxide (H_2O_2) and NH_3 .

We have previously described that MET has a pharmacological role in modulating food intake in rodents. In particular, MET injected into the central nervous system of rats presented hyper- or hypophagic effects depending on the dosage injected. In particular, the hyperphagic effect was mediated by increased hypothalamic levels of nitric oxide (NO) (Raimondi *et al.*, 2007). However, due to the experimental model, we could not conclude on whether MET effect on NO was direct or mediated by the release of other neurotransmitters.

Aim: The aim of this work was to explore whether MET could directly increase NO levels. To this we studied the effect of exposing rat white adipocytes, cell expressing either Bz-SSAO or a complete NO system, to MET (from 0.5 to 10 μM).

Methods: Nitrite/nitrate (NO_x) levels were measured amperometrically as an indirect measure of NO levels in cell medium. Nitric oxide synthase activity was measured radiochemically (Gallo *et al.*, 1998) and cGMP by an indirect ELISA method.

Results: MET increased, in a concentration-dependent fashion, NO levels in adipocyte medium reaching the maximum effect at 10 μM ($0.51 \pm 0.15 \text{ nmol}/10^4 \text{ cells}/30 \text{ min}$; $n=15$). This effect was inhibited by pre-incubating cells with L-NAME (10^{-4}M) or alpha amino guanidine (10 mM), by nisoldipin (10^{-6}M), a selective blocker of L-type Ca^{++} channels and by removing extracellular Ca^{++} . MET also increased cell cGMP ($27.6 \pm 6.5 \text{ pmol}/10^4 \text{ cells}/15 \text{ min}$ for MET 5 μM ; $n=5$). Interestingly, inhibition of Bz-SSAO by MDL72223 further increases NO cell medium levels.

Conclusions: MET can increase directly NO levels in isolated cells. MET effect is the result of the stimulation of the Ca^{++} -dependent nitric oxide synthase activity, likely the

NOs3. Moreover these results suggest that the insulin-like effects of Bz-SSAO substrates and the anti-inflammatory activities of Bz-SSAO inhibitors might include the NO signaling.

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P10

Mechanisms of the analgesic effect of corticotropin-releasing factor on the somatic pain sensitivity in rats

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Corticotropin releasing factor (CRF) and its receptor subtypes (CRF-1 and CRF-2) have been implicated in the regulation of physiological functions and behavior in stress. Stress is known to cause analgesia. Activation of CRF-producing neurons by pain and the analgesic effect observed after CRF administration provide evidence of the involvement of CRF in stress-induced analgesia. The aim of the present work was to investigate the mechanisms of the analgesic effect of CRF on the somatic pain sensitivity.

Electrical current threshold test (Bogdanov et al., 2003) was used for measurement of the somatic pain sensitivity. The pain threshold was the minimum current necessary to provoke tail withdrawal reaction. Systemic administration of CRF (40 µg/kg, i.p.) induced rapidly developing (from 3 min) and long-lasting (30 min) increase in the pain response threshold (the analgesic effect). The CRF-induced increase in the pain response threshold was accompanied by an increase in the plasma corticosterone level. To understand the mechanisms of CRF-induced analgesia we investigated the contribution of hypothalamic-pituitary-adrenocortical (HPA) axis (which is activated by CRF through CRF-1 receptor subtype) as well as the involvement CRF-2 receptor subtype and opioid system in the analgesic effect of CRF. The contribution of HPA axis in CRF-induced analgesia was studied by two approaches: pharmacological suppression of HPA axis activity and occupation of glucocorticoid receptors by its antagonist RU 38486. The opioid antagonist naltrexone and CRF-2 receptor antagonist astressin 2-B were used for examination of the involvement of opioid system and CRF-2 receptor subtype in CRF-induced analgesia.

Pretreatment by naltrexone had no effect on both CRF-induced analgesic effect and an increase in plasma corticosterone. Although CRF-2 receptor antagonist astressin 2-B did not change CRF-induced plasma corticosterone increase,

it completely abolished CRF-induced analgesia. Pharmacological suppression of HPA axis, leading to the inability of the system to increase the hormone levels (CRF, ACTH, corticosterone), resulted in a decrease in the degree and duration of CRF-induced analgesic effect. CRF-induced analgesic effect was partly abolished by glucocorticoid receptor antagonist RU-38486.

The data obtained suggest that the analgesic effect of CRF on somatic pain sensitivity in our experimental conditions was mediated by nonopioid mechanisms. CRF-induced analgesia may be provided by both: 1) mechanisms associated with HPA axis and mediated through glucocorticoid receptors and 2) mechanisms not associated with HPA axis and mediated through CRF-2 receptors.

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P11

Inhibition of receptor activator NF-κB (RANKL) induced osteoclast formation by histone deacetylase inhibitors

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High expression of RANKL by inflammatory cells is associated with bone loss in several common inflammatory diseases including rheumatoid arthritis and periodontal disease (Crotti et al., 2003; Crotti et al., 2002; Haynes and Crotti, 2003). Histone deacetylase (HDAC) inhibitors are a novel class of drugs that play a role in regulation of gene transcription. HDAC inhibitors have been identified as anti-inflammatory agents and anticancer agents (Johnstone, 2002). A recent report suggests that they may inhibit osteoclast formation from a murine cell line (Nakamura et al., 2005). The aim of this study was to investigate effects of novel HDAC inhibitors in an *in vitro* osteoclast formation assay.

Osteoclasts were generated over 14 days culture from peripheral blood mononuclear cells (PBMCs) in the presence of HDAC inhibitors, RANKL and other factors. Osteoclast formation was determined by resorption of dentine substrate.

The results of this study showed that both drugs inhibited osteoclast bone resorption *in vitro*. The novel class I and II HDAC inhibitor, 1179.4b, was the most effective significantly ($p < 0.05$) reducing resorption at concentrations of 0.16 nM

and higher. MS-275, a previously investigated selective class I inhibitor, was found to significantly ($p < 0.05$) inhibit osteoclast resorption at concentrations of 20 nM and above.

These drugs, therefore, may have potential to inhibit the enhanced osteoclast bone resorption associated with several common chronic inflammatory diseases.

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P12

Cannabinoid receptor activation induces apoptosis in colon cancer cells through TNF α and ceramide

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Cannabinoids have been recently proposed as a new family of potential antitumor agents. This antitumor action relies on the ability of these drugs to inhibit tumor angiogenesis (Blazquez *et al.*, 2004) or directly induce apoptosis or cell cycle arrest in neoplastic cells (Galve-Roperh I *et al.*, 2000; Blazquez *et al.*, 2006). Although it is well known that the endogenous cannabinoid system and cannabinoid receptors regulate gastrointestinal functions such as gastric emptying, secretion and intestinal motility (Massa *et al.*, 2006), few studies investigated the expression and role of CB₁ and/or CB₂ receptors in normal (Wright *et al.*, 2006) or neoplastic (Cianchi *et al.*, 2004) human colon epithelial cells.

The present study investigates the expression of cannabinoid receptors, CB₁ and CB₂, in colorectal cancer and provides new insight into the molecular pathways underlying the anti-inflammatory and apoptotic effect induced by their activation.

Cannabinoid receptor expression was investigated in both human cancer specimens and in the DLD-1 and HT29 colon cancer cell lines. The effects of the CB₁ agonist ACEA

and the CB₂ agonist CB13 on TNF- α production, ceramide and tumor cell apoptosis were evaluated. We used a selective small interfering RNA to *knockdown* TNF- α mRNA.

We show that the CB₁ receptor was mainly expressed in human normal colonic epithelium whereas tumor tissue was strongly positive for the CB₂ one. Activation of the CB₁ and, more efficiently, of the CB₂ receptors, reduced inflammation, increased ceramide levels and induced apoptosis in DLD-1 and HT29 cells. Apoptosis was prevented by pharmacologic inhibition of ceramide *de novo* synthesis. The CB₂ agonist CB13 also reduced the growth of DLD-1 cells in a xenograft mouse model of colon cancer. *Knockdown* of TNF- α mRNA abrogated the increase in ceramide and therefore, the apoptotic effect induced by cannabinoid receptor activation.

In conclusion the present study demonstrates that either CB₁ or CB₂ receptor activation induces apoptosis through ceramide *de novo* synthesis in colon cancer cells. Our data unveiled, for the first time, that TNF- α acts as a link between cannabinoid receptor activation and ceramide production.

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P13

Human whole blood assay for rapid and routine testing of non-steroidal anti-inflammatory drugs (NSAIDs) on cyclo-oxygenase-2 activity.

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Drug development programs for cyclooxygenase-2 (COX-2) selective inhibitors are still ongoing despite the recent toxicity problems associated with Vioxx[®]. Thus, there is still a need for reliable and easy to handle COX-assay systems. Among several *in vitro* testing systems the whole blood assay (WBA) is a well known method to examine non-steroidal anti-inflammatory drugs (NSAIDs) in view of their potency to inhibit COX activity. This assay has some major advantages over enzyme-based or isolated cell assays. Emergence of artifacts due to cell separation steps is kept to a minimum and substances, even in unproportionally high concentra-

tions, can be examined outside the body in a physiological environment resembling most closely the *in vivo* conditions in living humans, i.e. 37 °C, homeostasis, all blood compounds are present and cell-cell interactions remain intact. While human whole blood assays for COX-1 are performed within less than two hours, for established COX-2 assays one still has to allow for an over night incubation step before obtaining the desired plasma. The aim of this study was to characterize an optimized, fast and simple human whole blood assay (hWBA).

The assay includes elimination of COX-1-activity using ASS (a selective COX-1 inhibitor) and the thromboxane synthetase inhibitor (TXBSI), COX-2 induction via LPS and measurement of PGE₂. Using TXBSI reduces the amount of LPS and results in higher prostaglandin production.

Cremophor EL[®]-EtOH was used as vehicle instead of DMSO because within a defined concentration range, Cremophor EL[®]-EtOH allows even very hydrophobic drugs to be solubilized and applied *in vitro* without cell damage. Cremophor EL[®]-EtOH at 0.2 % was optimal as at this relatively low concentration excellent drug dissolution was obtained whereas many hydrophobic substances precipitate in 0.2 % DMSO.

P14

Effects of nimesulide on drg neurons: Inhibition of pkc-ε translocation and of substance P synthesis and release

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Nimesulide is a non-steroidal anti-inflammatory drug (NSAID) with a marked analgesic activity. We examined if this drug was able to modulate the activity of peripheral nociceptors stimulated with inflammatory mediators in culture and in an *in vivo* model of inflammatory pain. We quantified the number of cultured neurons isolated from rat dorsal root ganglia (DRG) showing translocation of PKC-ε caused by exposure to 1 mM BK or 100 nM thrombin for 30 seconds, using immunocytochemistry, in control conditions and in the presence of different concentrations of nimesulide (0.1, 1.0 and 10 mM). Translocation was observed with a confocal microscope. We also measured the level of substance P (SP) released by DRG neurons and the level of preprotachykinin mRNA expression in basal conditions and after 36 hours of stimulation with NGF or with an inflammatory soup containing 1 mM bradykinin (BK), 100 nM thrombin, 100 nM endothelin-1 and 25 mM KCl. Preprotachykinin mRNA was measured with real-time RT-PCR. SP released in the culture medium was measured with a radioimmunoassay (RIA) technique. Nimesulide significantly reduced the number of

DRG neurons showing BK- and THR-induced translocation of PKC-ε to the plasma membrane. Nimesulide (10 mM) also significantly decreased the mRNA levels of the SP precursor preprotachykinin, and decreased the amount of SP released in the medium during 36 hours of treatment with NGF or with the inflammatory soup. In *in vivo* studies inflammation was induced by the intraplantar injection of complete Freund's adjuvant (CFA, 0.1 mg/0.1 ml) in the rat hind-paw. The anti-hyperalgesic effect produced by the acute administration of nimesulide (5.0 mg/kg p.o.) 3 days after CFA injection was evaluated by the Randall-Selitto paw-withdrawal test. The intensity of hyperalgesia was measured at 30 and 60 minutes after drug administration. In addition, we investigated the effects of nimesulide on the production of SP in the inflamed hind-paw. Nimesulide significantly reduced both the inflammatory hyperalgesia to mechanical stimulation and the levels of SP in the inflamed paw.

Our data suggest that the analgesic effects of nimesulide are due at least in part to the reduction of the sensitisation of peripheral nociceptors caused by inflammatory mediators, and demonstrate an involvement of SP and PKC-ε in the analgesic actions of this NSAID. Taken together, our findings provide further evidence that nimesulide may be a particularly useful agent for the treatment of acute inflammatory pain.

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P15

Anti-rheumatic activity and cytokine synthesis inhibition by the oligosulphated disaccharide sucrose octasulphate

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Di-Sucrose octasulphate (SOS), an orally absorbable oligosulphated disaccharide (Hiebert et al, 2002), is released by gastric acid from the muco-adhesive anti-ulcer and anti-IBD drug sucralfate. We aimed to assess the anti-rheumatic potential of SOS.

Murine antigen induced arthritis (MAIA) was induced by sensitization and i.a. challenge with methylated bovine serum albumin. Rat (RCIA) and mouse (MCIA) collagen arthritis were induced by sensitisation to bovine collagen. Micro-CT images were acquired using a Siemens Microcat II instrument, scan duration 30 minutes. Images were reconstructed with 768 z slices each having 512 × 512 pixel resolution (32 mm × 32 mm). For isosurface plots, density thresholds were set by reference to intact mouse metatarsal bone (AMI-RA). The erosion of the metatarso-phalangeal joints was scored according to Mancini et al (2008). TNFα synthesis was assessed in PHA stimulated human whole blood (HWB)

in culture, LPS stimulated PMA differentiated U937 macrophages, murine J774 macrophages, and LPS stimulated IL2 synthesis by Jurkat T cells.

Sucralfate (100 mg/kg p.o. 1 hr. prior to challenge) significantly inhibited stifle joint inflammation at 24 hrs. Proton pump inhibition (lansoprazole) antagonised this activity indicating gastric conversion of sucralfate into an anti-rheumatic agent. SOS (30–100 mg/kg p.o. 1 hr prior to challenge) inhibited joint inflammation also. SOS (100 mg/kg days 0–35) significantly inhibited clinical signs and paw swelling in MCIA ($p < 0.01$), reducing the erosion of bone ($p < 0.07$). Administered over the sensitisation period, SOS inhibited RCIA clinical score and joint swelling (100 mg/kg p.o. days 0–11, $p < 0.01$). PHA stimulated HWB TNF α was inhibited ($IC_{50} < 0.1 \mu M$). LPS stimulated TNF synthesis by U937 cells was also ($IC_{50} < 0.1 \mu M$). TNF α inhibition in J774 cells only occurred at high concentrations ($> 100 \mu M$). IL-2 synthesis by PHA stimulated Jurkat cells was also attenuated ($IC_{50} < 2 \mu M$).

SOS is anti-rheumatic/inflammatory *in vivo*. This could be related to TNF and IL2 suppression. Heparin disaccharides suppress murine DTH and T cell TNF α (Lider et al, 1995), as well as adjuvant arthritis and macrophage TNF α (Cahalon et al, 1997), and T cell function (Hecht et al, 2004). SOS appears to act on both the sensitisation and challenge steps of arthritic disease.

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P16

Some *in vitro* actions of nitric oxide releasing non-steroidal anti-inflammatory agents on cervine and ovine digital artery rings

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There is much evidence showing that treatment with non-steroidal anti-inflammatory drugs (NSAIDs) can lead to gastric irritation and erosion associated with reduced secretion of protective prostaglandins and decreased mucosal blood flow (see Whittle, 2003). If mucosal blood flow could be increased and absorption enhanced, these unwanted actions

might be reduced. One possible approach was highlighted by Tashima *et al.* (2000) who showed that coupling a nitric oxide (NO) containing moiety to an NSAID (NO-NSAID), such as nitroxybutyl aspirin (NCX-4016; NO-aspirin) lead to a reduction in gastric irritancy.

A preliminary study was undertaken to see if part of the beneficial effect of NO-NSAIDs could be the result of increased blood flow removing the drug from the gastric mucosa more effectively. It was found that NO-aspirin significantly decreased the agonist-induced tension generated by the smooth muscle of ring segments of the common digital artery of fallow deer while aspirin and its butyl ester were virtually without effect (Benedict *et al.*, 2005)

In order to extend these observations further, arteries were obtained from the forefeet of fallow deer (*Dama dama*) of either sex (38–48 kg body weight) or from sheep of mixed breeds similar weights, killed under E.U. red meat regulations. Rings (2–3 mm) were mounted in 10 ml organ baths in Krebs-Henseleit physiological solution aerated with 95 % O₂ and 5 % CO₂ and subjected to a tension of 3.0 g. Control experiments where the responses of the rings to 5-hydroxytryptamine (5-HT) were compared with those of the whole perfused forelimb confirmed that the rings from a conductance artery were a reliable model of the responses of the whole vascular bed, which included the fine resistance vessels.

The actions of NO-aspirin and NO-ibuprofen (synthesized by Dr Akram Khan at Sheffield Hallam University) were then compared with their parent compounds on rings contracted by either 5-HT or by electrical field stimulation (EFS; 0.1–40 Hz, at just supra-maximal voltage, for 10 s every 100 s).

With 5-HT and with EFS, the results confirmed the earlier observations with NO-aspirin when compared with the actions of aspirin itself. Moreover, the action of NO-aspirin was unaffected by the presence of L-nitro-L-arginine methyl ester (L-NAME) but was inhibited by 1*H*-[1,2,4] oxadiazolo [4,3- α] quinoxalin-1-one (ODQ), strongly suggesting that its action was through the release of NO. Comparison of the actions of NO-aspirin (10⁻⁴ M) with the actions of the known NO-releasing agent, amino-3-morpholino-1, 2,3-oxadiazolium chloride (SIN-1 [10⁻⁴ M]) against the tension produced by 1 μM 5-HT showed that there was no significant difference in effect ($n=4$). The percentage of the maximum response to 5-HT in the presence of aspirin was 7 \pm 10 % and in the presence of SIN-1, it was 17 \pm 8 % compared with 95 \pm 2 % in the absence of either drug. This suggests that NO-aspirin, under the conditions employed in these experiments, is as active as SIN-1 as a source of NO.

In sheep arteries, both NO-aspirin and NO-ibuprofen produced concentration-related antagonism of both 5-HT and EFS-elicited contraction to a similar extent. However, when NO-ibuprofen and ibuprofen were compared in the deer digital artery rings, it was found that NO-ibuprofen was much less effective than ibuprofen. This was particularly the case with 5HT-contracted rings. With EFS pre-contraction, neither compound was effective.

So far, the antagonism of 5-HT-elicited contractions of deer digital arteries has been shown to be partially sensitive to mepyramine (pyrilamine), suggesting an involvement of H₁ receptors but it is also partially reduced propranolol and

ketoprofen. ODQ had no effect, indicating that it is unlikely that ibuprofen acts by releasing NO from the tissues.

Although no clear explanation is possible of the ibuprofen actions seen in the rings from deer, these results have exposed a difference in response between two species of ruminant herbivore that needs to be resolved.

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P17

Ribonucleate sodium [Osteochondrin S[®]] inhibits cytokine-induced cartilage-bone degradation but not proteoglycan synthesis

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Osteochondrin S [OST] comprises a mixture of ribonucleotides from bovine connective tissues (BSE-free accredited) and yeast and has been found to be effective in treatment of osteoarthritis (OA) and other musculo-skeletal conditions (Rainsford, 1996, Rainsford *et al.*, 2004).

Aims & Objectives: In order to gain insight into the mode of action of OST on cartilage and bone destruction we performed studies using bovine ankle and human articular cartilage in organ culture to establish if OST affects the proteoglycan destruction induced by the pro-inflammatory cytokines, interleukin-1 (IL-1) and tumour necrosis factor- α [TNF], whose key role in cartilage and bone destruction in OA is well-established.

Methods: The conditions for organ culture, measurement of proteoglycan (PrGn) breakdown as glycosaminoglycans (GAGs) released into the culture medium and radiol sulphate incorporation as a measure of PrGn synthesis were those as described (Rainsford *et al.*, 1989). Bovine cartilage-subchondral bone explants were incubated in DMEM+5%FCS for 3d in the presence of 0.1 $\mu\text{g.mL}$ IL-1 α and / or 0.5 $\mu\text{g.mL}$ TNF and 12.6, 63.0, 126.0 or 315 $\mu\text{g.mL}$ ribonucleate sodium [RNS] (equivalent to that as in OST). At completion of incubation the GAG content in digested cartilage and that in the medium was measured using the dimethylmethylene blue (DMB) technique. Human hip OA cartilage explants were incubated as above for 5d in 0.1 $\mu\text{g.mL}$ IL-1 α and 25.2,

126 or 630 $\mu\text{g.mL}$ RNS together with ³⁵sulphate. At the completion of incubation the GAG concentrations in the culture medium and the PrGns/GAGs remaining in the digested cartilage were determined using the DMB technique. The radioactivity in the isolated GAGs/PrGns in the cartilage and medium was determined by β -scintillation counting.

Results: OST treatment resulted in a concentration-dependent reduction in IL-1- and TNF-induced PrGn degradation in bovine cartilage-bone or cartilage as well as in IL-1- induced PrGn degradation in human tissues. Statistically-significant (ANOVA, t-test) reductions were observed with 63–316 μg RNS against IL-1 induced bovine cartilage degradation and 316 μg RNS against TNF degradation. More pronounced effects were observed on PrGn degradation induced by IL-1 than TNF in cartilage-bone compared with cartilage alone. Pre-incubation with RNS for 1d did not further enhance its preventive effects. In human tissues similar protection by RNS was observed against IL-1 induced cartilage degradation, but only at the higher concentration of 630 $\mu\text{g.mL}$ RNS. There were no significant effects of RNS on radiol sulphate incorporation into the isolated PrGn/GAG fractions.

Conclusions: RNS in OST has protective effects against cartilage destruction induced by two predominant pro-inflammatory cytokines that are responsible for joint destruction in OA. This suggests that OST may protect against joint damage in OA. However, studies in humans with this condition may be worth undertaking to establish if joint destruction is reduced in this condition when assessed using MRI and radiological methods.

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P 18

A novel compound from celery seed with a bactericidal effect against *Helicobacter pylori*

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As well as peptic ulcers, *Helicobacter pylori* is associated with the development of gastritis, gastric adenocarcinoma and lymphoma; it being classified as a class I carcinogen in humans (International Agency for Research on Cancer Working Group, 1994). Although the bacteria can be eradicated in up to 90% of patients, side effects, poor compliance and the resistance of the bacteria to antibiotics are common causes of frequent treatment failure. Celery seed extracts (CSE) from a unique source in India has been used a herbal medicine since antiquity and found to have anti-inflammatory and gastroprotective properties (Butters et al., 2004; Whitehouse et al., 2001). This study followed on observations that crude extracts of CSE exhibited anti-helicobacter activity (Rainsford & Liu, 2003).

CSE was selectively fractionated using organic solvents followed by HPLC. Fractions were collected and bio-assayed against different strains of *H. pylori* using conventional culture methods. The most potent component that was obtained from HPLC and purified was designated CAH. This compound had potent bactericidal effects against *H. pylori*; the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) being 3.15 µg ml⁻¹ and 6.25–12.5 µg.ml⁻¹, respectively. This compares favorably with the MIC and MBC of tetracycline, which are both 3.15 µg.ml⁻¹. The isolated compound had highly specific inhibitory effects on *H. pylori*, since no inhibitory activity was detected against *Campylobacter jejuni* or *Escherichia coli* at these levels. The molecular ion of CAH was measured as 384.23 by mass spectrometry (using peak matching), giving the empirical formula as C₂₄H₃₂O₄. The MS and NMR data strongly suggest this compound is a phthalide dimer. From radioactive bioassay, CAH inhibits RNA synthesis by 50% of that seen in a negative control in 3 days, while DNA and protein synthesis was unchanged.

In conclusion, (1) CAH is a novel component from celery seed with effective and specific inhibition against *H. pylori*; (2) there are novel anti-*H. pylori* mechanisms and there is no evidence of resistance; (3) it has potential for safer use and a low cost therapy; (4) it may avoid the alterations in GI flora caused by using antibiotics. These results suggest that the new compound may be suitable for further investigation as an agent for treating *H. pylori* infections.

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P19

Therapeutic colloidal silver: time for re-appraisal?

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Background: Colloidal silver (CS) was introduced as an oral/injectable/topical antiseptic almost 100 years ago; being used to combat infectious diseases before sulphonamides (1930's) and antibiotics (1940's) became available. Today the Internet provides many ill-proven claims for CS efficacy and advertising for CS generators. Both commercial and home-produced CS preparations contain soluble ionic silver, Ag(I), along with colloidal metallic silver (CMS) nanoparticles. They may also contain finely dispersed silver oxide and even silver "peroxide" (an Ag(I), Ag(III) oxide). Thus one CS product may contain four or more species of silver, differing in efficacy/toxicity. Uncritical use of impure silver e.g. silver coinage, to prepare CS introduces other components/toxins such as copper, lead or cadmium salts/oxides.

Objectives: (i) to examine the variability and composition of some CS preparations sold in Australia; and (ii) to quantify possible benefit of oral CMS for promoting wellness, by capacity to suppress arthritis development in rats.

Results: (i) The ratio of CMS/total silver in some purchased preparations varied from <10% to >50%; and (ii) A well-defined CMS (Lunasol™), given orally 7 times/2 weeks for a cumulative silver dose of 0.6 mg/kg to Wistar or DA rats, inhibited development of the adjuvant-induced polyarthritis. Silver acetate and silver oxide were ineffective at twice this dose. However, arthritis slowly developed after ceasing treatment; also seen after ceasing daily prophylactic NSAID treatment (150 mg/kg aspirin, or 80 mg/kg ibuprofen, or 50 mg/kg naproxen). By contrast oral CMS given as monotherapy did not prevent development of collagen-induced arthritis in these rats. Similar differential anti-arthritic activity was also observed after parenteral administration of the gold drug, sodium aurothiomalate (Mycrocrisin®) at a cumulative dose of 20 mg/kg.

Conclusion: CMS as nanoparticulate silver metal hydrosols may be a useful adjunct therapy when freed of toxic ionic silver, perhaps acting like a regulatory 'immunobiotic' (Clancy, 2003) to promote wellness. A classic probiotic *Lactobacillus* GG bacteria likewise suppresses adjuvant-induced arthritis in rats. (Baharav et al 2004).

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P20

Is the pathogenesis of “binge drinking” an inflammatory-mediated process?

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There is wide concern at the increasing occurrence of “binge drinking”, particularly in adolescence, which causes severe cognitive impairment in susceptible individuals. The pathogenesis of this lesion remains unclear but may be due to changes in immunological function, which leads to microglia in specific brain regions.

Female rats (100 g at the commencement of the study) were subjected to a regime of “binge drinking” for 3 weeks, as previously described (Ward et al., 2008). A range of amino acids were then assayed in the CA1 hippocampal region, both before and after a further dose of ethanol, by microdialysis and HPLC (Bianchi et al., 1999). At the end of the experiment, phagocytic cells (alveolar macrophages) were isolated from each rat, and their ability to release inflammatory cytokines, before and after *in vitro* stimulation with lipopolysaccharide, assayed. Rat brains were removed, fixed in 4% paraformaldehyde and the activation of microglial cells within specific brain regions determined by OX6 staining.

Binge drinking regime, either 2 g/kg or 3 g/kg, enhanced the basal level of glutamate in the CA1 hippocampus, approximately 2 fold, although a further dose of ethanol had little effect on the levels of glutamate, aspartate, taurine and GABA. The macrophages isolated from rats administered either a 2 g/kg or 3 g/kg ethanol binge drinking regime, exhibited a significant increase in iNOS prior to stimulation, as reflected by increased NO release. Furthermore, after stimulation there was a statistically significant (ANOVA, $P < 0.05$) elevation of the LPS-stimulated NO release.

Such results suggest that the pathogenesis of “binge drinking” may be mediated via the increased release of the excitotoxic neurotransmitter glutamate, which activates microglia to release pro-inflammatory mediators.

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P 21

p38 MAP kinase triggers dendritic or accessory cells in the allogeneic mixed lymphocyte and Con-A-induced lymphocyte responses

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The European Union KINACEPT programme is a new initiative investigating the role of p38 MAPKinase in rheumatoid and inflammatory bowel disease. p38 mediates TNF α synthesis, and p38 inhibition in dendritic cells (DCs) is reported variously to have positive and negative results, depending on time, place and differentiation state. We have investigated the inhibition of p38 MAPKinase with a new selective inhibitor, ML3403 on DC accessory cell function in the mixed lymphocyte reaction (MLR) and non-specific accessory cell function.

For the MLR C57Bl6 mouse bone marrow (BM) or spleen (Sp) were GM-CSF differentiated, T cells were isolated from Balb/c mice. SpDCs or BMDCs were stimulated +/- 100 ng/ml LPS. T cells were added for 5 days. Cell mixtures were incubated, or DCs underwent 2 hours preconditioning, with ML3403 ((RS)-{4-[5-(4-Fluorophenyl)-2-methylsulfonyl-3H-imidazol-4-yl]pyridine-2-yl}-(1-phenylethyl) amine, ML). 3H-thymidine incorporation was measured, cell viability by MTT assay, TNF α and IL-12 production by ELISA.

For non specific reactions Balb/c mouse spleen cells were stimulated with 2.5 μ g/ml Con-A in the presence or absence of 3 μ M ML3403 and incubated for 72 hours. Accessory cells (AC) were separated from lymphocytes. Separated cells were pre-conditioned with drugs for 2 hours, washed 3 times, re-suspended (1×10^6 /mL), mixed and stimulated with Con-A. Con-A induced lymphocyte proliferation was dependent on ACs.

MLR T cell proliferation induced by allogeneic SpDCs or BMDCs was inhibited by ML3403 (IC₅₀ SpDC 0.03 μ M, BMDC 0.03 μ M). 2 hour preconditioning of DCs with 3 μ M ML3403 followed by washout had a subsequent inhibitory effect, but reduced LPS stimulated IL-12 and TNF α synthesis at lower preincubation concentrations (IC₅₀ IL-12 0.9 μ M, TNF 0.11 μ M). p38 plays a role in the interaction of DCs and T cells in antigen recognition. In addition, steady state p38 activity is required prior to DC activation.

ML3403 (3 μ M) also inhibited Con-A induced whole spleen cell proliferation. 2hr preconditioning of ACs with ML3403 followed by washout inhibited AC induced lymphocyte proliferation, whilst preconditioning of lymphocytes was without effect. ACs as well as DCs thus require functional steady state p38 activity prior to allogeneic or Con-A

stimulation in order to stimulate lymphocyte proliferation. Preconditioning of accessory cells with a p38 inhibitor results in long lived inhibition of accessory cell function after removal of the drug. Thus, p38 inhibitors may thus possess extended pharmacodynamic properties after metabolism.

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A simple score for assessing bone erosion in rodent arthritic paws visualised by micro focal Computer Tomography X-ray

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Abstract: The EU KINACEPT programme is investigating mechanisms behind joint erosion in rheumatoid arthritis as part of its Drug Discovery program. Rodent arthritis models result in joint destruction that traditionally requires observer scoring of histological sections. Micro-CT systems linked with sophisticated software can now visualise entire paws in three dimensions. However, even now, quantitative analysis of the multiple joints found in the paw remains difficult and labour intensive. Algorithmic calculations can be used to

calculate indices such as bone surface roughness (Silva et al., 2006), bone size (Silva et al, 2004, Proulx et al, 2007), and bone cortical volume (Barck et al., 2004) Whilst such systems are being developed to improve experimental throughput, we have devised a visual score for the quantitative determination of bone erosion in whole paws, termed the S&M (Seed & Mancini) score.

Collagen-induced arthritis was induced in male dba mice. Arthritis was scored arthritis, with each affected digit and ankle/wrist=1, max=22, and hind paw inflammation plethysmometry. Paws were taken at 32–33 days and fixed in formal saline. Micro-CT images were acquired using a Siemens Microcat II instrument, scan duration 30 minutes. Images were reconstructed with 768 z slices each having 512 × 512 pixel resolution (32 mm × 32 mm). For isosurface plots, density thresholds were set by reference to intact mouse metatarsal bone (AMIRA). The erosion of the metatarso-phalangeal joints was scored as follows: 0 = Anatomically Normal; 1 = Point erosion Metatarsal; 2 = as 1, + elongated erosion; 3 = as 2, + complete penetration; 4 = as 3 + elongated penetrative eroded meta-tarsal, erosion in proximal phalangeal bone; 5 = as 4 + complete penetrative erosion of proximal phalangeal bone; 6 = as 5, + metatarso-phalangeal joint destroyed. The scores for each of four digits are summed to give a maximum of 24.

One mouse of 22 did not acquire arthritis and was excluded. D'Agostino & Pearson omnibus normality test showed the distribution to be not normal (K2=19.46 (p<0.0001). Mice elicited a median S&M score of 9 (IQR 0–20.5, mean 10.6 ± s.e.m. 2.1, n=21). Scatter plot showed the presence of responders (S&M score >3) and non-responders. Responder median 20 (IQR 12.5–21.8, mean 17.9 ± s.e.m. 1.7, n=12). There was no correlation with clinical score (Spearman r = -0.11, p=0.65, n=21), or paw volume (Spearman r = -0.16, p=0.49, n=21). Treatment with 0.1 mg/kg Dexamethasone significantly reduced the erosion from median 11 (IQR 0–20, n=11) to 1 (IQR 0–5, p<0.01 Fishers Exact Test, n=11).

Despite dba mice being an inbred strain, their erosive disease splits between two populations. This project will analyse these differences with respect to p38, cytokine and chemokine protein and gene expression over time.



Score 0

Score 11

Score 24

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