



Inflammo
PHARMACOLOGY
An International Journal of
Inflammation and Pharmacology

20 years of Inflammopharmacology

Celebrating Leading Contributors and the
Journal's Place in the Development of the Field

Department of Pharmacology, University of
Cambridge, Cambridge, UK
4th – 6th July 2011

PROGRAMME
and
ABSTRACTS

International Conference on

20 years of *Inflammopharmacology*

INTRODUCTION & WELCOME

This is the 10th in the series of the Inflammopharmacology Conferences, which initially started in 1982. The range of topics covered at the meetings has varied according to the prevailing issues at the time concerning the safety and efficacy of anti-inflammatory drugs and approaches for treating pain and inflammatory diseases.

This meeting celebrates 20 years of publication of the international journal, *InflammoPharmacology*. The content of the conference has been planned to examine not only the current developments in these fields, but also at what may be regarded as future trends and possible innovative areas for research in the future. These ideas may be useful for planning the direction of subject areas in the Journal in future.

This meeting would not have been possible without the financial support and generosity of the participants, many of whom have given support by paying travel and accommodation. We thank Dr Detlef Klüber and the publishers of *InflammoPharmacology*, Springer (Basel) AG (Basel, Switzerland) for generous financial and general support. We also thank Dr Ron Zimmerman and PLx Pharma (Houston, Texas, USA) and IBSA (Lugano, Switzerland) for support of speakers.

We specially thank the Head of the Department of Pharmacology, University of Cambridge, Professor Peter McNaughton, for his support in donating use of departmental facilities, and the staff of this Department for their valuable help and generosity. Also, our thanks to the staff of Queens' College, Cambridge, for their help and valuable advice.

We would like to record our appreciation for the help and support of Mrs Veronica Rainsford-Koechli, Mr Alexander Rainsford. KDR would like to express sincere thanks for the kind hospitality provided by Dr Brian and Mrs Margaret Callingham.

The content of this programme is entirely the responsibility of the organizers.

Professor Kim D Rainsford
Dr Brian A Callingham
Conference Organizers

Inflammo **PHARMACOLOGY**

An International Journal of Inflammation and Pharmacology

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International Conference on

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CONFERENCE PROGRAMME

Tuesday 5th July 2011

Conference Theme: *Celebrating the research and contributions of some leading researchers*

Format of Presentations: Lectures/discussion led by invited speakers (listed below) for about 15-20 mins outlining key points, followed by brief questions relating to the presentations with a broader "round table" discussion among all participants at end of each "themed" session. Abstract numbers for Speakers (S) and Posters (P) next to titles.

Registration: 4-6pm – Monday July 4th, at Queens' College
8.40 am - Tuesday July 5th, at the Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge.

Introduction

Chairmen: Dr Brian Callingham, Prof Kim Rainsford

9.10 - 9.20: Professor Kim Rainsford – Editor, *Inflammopharmacology*
Wellcome and introduction.

Historical perspective and future of Inflammopharmacology as a research discipline

9.20 - 9.45: Professor Peter McNaughton
Ion channels underlying inflammatory and neuropathic pain [S1]

Theme: GI Ulcers, Acid, NSAIDs and Anti-ulcer Therapies

9.45 – 10.05: Professor Richard Hunt, McMaster University, Hamilton (ON), Canada
Advances in Understanding Inflammation in Upper GI diseases [S2]

10.05 – 10.30: Professor Ingvar Bjarnason, King's College Hospital, London, UK
Pitfalls in concepts of the roles of prostaglandins in GI effects of NSAIDs [S3]

10.30 – 10.55: Professor Klara Gyires, Semmelweis University, Budapest, Hungary
Central neural pathways mediating gastroprotection [S4]

10.55 – 11.15: Coffee/tea

11.15 – 11.30: Discussion on themed issues

Theme: Therapy of Pain & Rheumatic Diseases

Chairmen: Prof Walter Kean, Prof George Nuki

11.30 – 11.55: Dr Ann Parke, St Francis Hospital & Medical Center, Hartford, CT, USA
Critical assessment of therapies for SLE and related diseases [S5]

11.55 – 12.20: Dr Andrew Östör, Addenbrooke's Hospital, Cambridge, UK
Evaluation of biologics in rheumatoid arthritis [S6]

12.20 – 12.45: Professor Garry Graham, St Vincent's Hospital, Sydney, NSW, Australia
The place of therapy with analgesics [S7]

12.45 – 13.10: Professor Nicola Volpi, University of Modena & Reggio Emilia, Modena, Italy
Chondroitin sulphate as a model for natural therapy of osteoarthritic disease [S8]

13.10 – 13.20: Discussion on themed issues

13.20 – 14.00: Lunch & Poster Viewing

Theme: New Insights and Future Prospects for New Therapies

Chairmen: Dr Michael Powanda, Dr Michael Seed,

14.00 – 14.25: Professor Stefan Laufer, Universität Tübingen, Tübingen, Germany
Novel agents for controlling inflammation [S9]

14.25 – 14.50: Dr John Gillard, Aegera Inc, Montreal, PQ, Canada
IAP inhibitors as novel agents for control of inflammation and cancer [S10]

Chairmen: Prof Richard Hunt, Dr Ann Parke

14.50 – 15.15: Dr Michael Powanda, Mill Valley, CA, USA
Conditional pharmacology, pharmacogenomics, epigenetics: issues for drug development and use [S11]

15.15 – 15.40: Professor George Nuki, Queen's Medical Research Unit, University of Edinburgh, Edinburgh, Scotland
Future therapy for gout [S12]

15.40 – 16.05: Professor Lenard Lichtenberger, University of Texas Health Science Center, Houston, TX, USA
Phospholipid complexes of NSAIDs as GI safer drugs [S13]

16.05 – 16.30: Professor Ludmila Filaretova, St Petersburg, Russia
Understanding how to make glucocorticoids GI safe [S14]

16.30 – 17:00: Tea/coffee & Poster Viewing

17.00 – 17.30: Discussion on themed issues - including that of poster presentations

17.30 – 18.00: Professor Richard Hunt, McMaster University, Hamilton (ON), Canada
Special Lecture: *Science, Society and "the Ologies": The Influence of Politics, Pharma and Professors.*

Poster Presenters should be by their posters to answer queries during the latter part of lunch and especially at the afternoon coffee/tea break. The topics raised in these will be used as key discussion points in the main meeting.

A report on this conference and the main conclusions from discussions will be published in Special Themed Issue of *Inflammopharmacology* along with submitted papers from contributors on the topic "The Future of Inflammation - Pharmacology".

ABSTRACTS SPEAKERS

S1

ION CHANNELS UNDERLYING INFLAMMATORY AND NEUROPATHIC PAIN

Peter A. McNaughton, Dept of Pharmacology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1PD

One of the distinguishing characteristics of the sensation of pain is that it is increased by inflammatory mediators such as prostaglandin E₂. The frequency of firing in nociceptive neurons signals the intensity of pain, and PGE₂ enhances the sensation of pain by increasing the frequency of action potential firing in response to a given level of painful stimulus. The enhanced firing is abolished by a blocker of the I_h inward current, suggesting an involvement of the HCN channel isoform family in modulating the frequency of action potential firing.

Genetic deletion of HCN1 ablates the fast-activating I_h which is seen in large neurons and in a small sub-population of cold-sensitive neurons, but leaves unaffected the slowly-activating I_h seen in small nociceptive neurons. Deletion of HCN2, on the other hand, reduces I_h in small neurons; the remaining current is insensitive to cAMP and probably reflects expression of the cAMP-insensitive HCN3. Significantly, deletion of HCN2 abolishes the effect of PGE₂ in accelerating action potential discharge.

In behavioural experiments on mice in which a deletion of HCN2 has been targeted to nociceptive neurons we find that inflammatory heat pain is abolished. Remarkably, neuropathic pain caused by a nerve lesion was absent when HCN2 had been deleted. Our results suggest that HCN2-selective blockers may have potential in alleviating inflammatory and neuropathic pain.

S2

ADVANCES IN UNDERSTANDING INFLAMMATION IN UPPER GI DISEASES

R H Hunt, C Y Yuan. McMaster University Health Science Centre, Hamilton, ONT, Canada

Erosive esophagitis (EE) and non-erosive reflux disease (NERD) are key components of reflux disease. Esophageal acid exposure initiates symptoms and induces or perpetuates mucosal damage. Diagnosis is based on typical reflux symptoms, endoscopic findings, pH monitoring, and histological changes (Vakil 2006, Modlin 2009).

The pathophysiology is complex involving acid, pepsin, bile and pancreatic secretions in the refluxate; failure of anti-reflux mechanisms and esophageal mucosal defense mechanisms. Weakly acidic reflux (pH 4-7) contributes to symptom generation but the precise role of acid in NERD remains unclear (Wang 2008). Mechanoreceptor-mediated pathways and different types of receptors are involved in symptom generation in NERD and peripheral and / or central hypersensitivity mechanisms contribute to symptoms (Modlin 2009). Esophageal sensory control is governed via vagal and spinal pathways. Neurally mediated effects of reflux and inflammation in the mucosa and submucosa can lead to neural changes including remodelling (Shaker 2007). Mucosal changes (erosions / breaks) are assessed by endoscopy

and the apparently “normal” esophagus may be neither normal nor healthy. The introduction of novel endoscopic and ultrastructural techniques indicate, minimal changes as potentially specific findings (Modlin 2008)

Histologic markers of reflux-induced mucosal injury are seen in most patients with NERD but have been considered of limited diagnostic value. Minimal changes, include basal cell hyperplasia, papillary elongation, neutrophil / eosinophil intraepithelial infiltration and dilatation of intercellular spaces (DISs). (Vieth 2008a, Zentilin 2005) which are responsive to PPIs, supporting their clinical relevance (Vieth 2008b). New categories of M (minimal) and Grade N are proposed modifications to the LA system. DISs occur in 41 - 100% of NERD patients vs. 0 to 30% of controls (Tytgat 2008). Acidification of intercellular spaces can trigger symptoms, suggesting a mechanism for enhanced perception of acid reflux in NERD patients (Caviglia 2007). Acid-sensing ion channels (ASICs) in neurons are proton-gated cation channels in peripheral sensory / central neurons, playing an important role in physiological and pathological conditions (Xiong 2008). Deleting the ASIC3 channel in a rodent model prevents gastritis induced acid hyper-responsiveness of the stomach-brainstem axis (Wultsch 2008).

The inflammation in GERD is complex and cytokine profiles may explain the different outcomes in reflux disease, EE, NERD, Barrett’s metaplasia etc. (Rieder 2010). Cytokine-mediated mechanisms are related to acid / peptic injury. Acidified bile salts significantly increased secretion of IL-8 and interleukin-1 β by squamous cells and media from these cells increased migration of T cells and neutrophils (Souza 2009). Interleukin (IL)-1 β and IL-8 expression correlates with histo-morphological changes in the esophageal mucosa of patients with EE and NERD and a stepwise increase of DISs and basal cell hyperplasia from controls, NERD towards EE. Gene expression of both cytokines correlated with histology (Monkemuller 2009). The transient receptor potential vanilloid-1 (TRPV1) receptor is implicated in acid induced inflammation and nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) are associated with up-regulation of TRPV1 receptors in patients with oesophagitis (Shieh 2010). Proteinase-activated receptor-2 (PAR-2) is elevated in patients with NERD and EE and induces pro-inflammatory and neuro-inflammatory effects altering trans-epithelial resistance and mediating visceral hypersensitivity (Kandulski 2010). Esophageal inflammation may be induced by PAR2 activation by the induction of NFkappaB- and AP-1-dependent IL-8 production (Yoshida 2007). Moreover, luminal bacteria in the esophagus are potentially important. Alterations in the oesophageal microbiome are associated with inflammation and intestinal metaplasia of the distal esophagus and a type II pattern contains a greater proportion of gram-negative anaerobes / microaerophiles primarily correlated with EE and BE (Yang 2009).

Caviglia R, et al. *Aliment Pharmacol Ther* 2007; 25:629–636.

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Shaker R. *J Clin Gastroenterol*. 2007;41 Suppl 2:S160-2.

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Souza RF, et al. *Gastroenterology*. 2009;137(5):1776-84.
Tytgat GN. *Curr Gastroenterol Rep*. 2008;10(3):231-4.
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Xiong ZG, et al. *Curr Opin Pharmacol*. 2008; 8(1):25-32.
Yang L, et al. *Gastroenterology*. 2009;137(2):588-97.
Yoshida N, et al. *Int J Mol Med*. 2007;19(2):335-40.
Zentilin P, et al. *Am J Gastroenterol*. 2005;100(10):2299-306.

S3

PITFALLS IN CONCEPTS OF THE ROLES OF PROSTAGLANDINS IN GI EFFECTS OF NSAID'S

Ingvar Bjarnason.

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The concept that NSAIDs cause gastrointestinal damage by their action to inhibit Cyclooxygenase (COX)-1, resulting in decreased mucus and bicarbonate secretion and reduced micro-vascular blood flow, is simple, logical, plausible and considered by many "proven" by the demonstrated safety of COX-2 selective agents. Are there any problems in accepting this?

During the wave of enthusiasm that followed the discovery that COX inhibition accounted for the therapeutic effects of NSAIDs and subsequently the COX-2 story almost all analyses of studies exploring alternative mechanisms of damage was spurned with contempt. Yet the COX hypothesis could not account for certain important clinical observations in man. Hence it was possible to decrease gastric prostaglandins by over 90% without damage. COX-inhibition or mucosal prostaglandin levels could not account for the different severity of short-term endoscopy damage in volunteers or the propensity of NSAIDs to cause serious GI bleeding. Furthermore the most highly selective COX-2 inhibitors (etoricoxib) caused more gastric damage than rofecoxib or celecoxib. However the COX dependent GI damage hypothesis is most firmly challenged in studies of COX-1 and 2 knockout animals.

In summary: COX-1 knockout animals that have mucosal prostaglandin levels less than 5% of wild type animals do not develop GI damage spontaneously and have a normal life expectancy. A selective COX-1 inhibitor (SC-560) reduces mucosal prostaglandin levels to a similar extent in wild type animals and similarly does not cause GI damage. Celecoxib, which does not cause GI damage in the short term in wild type animals causes characteristic NSAID damage in COX-1 knockouts. Interpretation: Dual inhibition of the COX enzymes leads to GI damage (Wallace *et al.* 2000, Sigthorsson *et al.* 2002)

However, COX-2 knockouts (50%) and long term celecoxib is associated with significant small bowel damage despite normal mucosal prostaglandin levels. Healthy COX-2 knockouts develop GI damage with SC-560. These findings suggest that COX-2 and not COX-1 is the housekeeping enzyme in the GI tract that is essential for maintaining GI integrity (Sigthorsson *et al.* 2002). This lends further support for the idea that dual inhibition of the enzymes is a pathogenic process in the damage.

Nevertheless this is not the full story. Concomitant inhibition of nitric oxide synthase and COX-2 leads to intestinal damage (Ohno *et al.* 2004) and COX-2 knockout animals exposed to "topical" injury develop identical damage to conventional NSAIDs (Hotz-Behofsits *et al.* 2010).

In conclusion, these studies clearly show that inhibition of COX-1 with decreases in mucosal prostaglandins, is at best just one of the mechanisms involved in the pathogenesis of NSAID-

induced gastrointestinal injury. Indeed COX-2 seems to play a greater part in the damage and most importantly NSAID-induced gastrointestinal damage can be caused by agents that do not decrease mucosal prostaglandin levels. The importance of the “topical” actions of NSAIDs, namely their effect on mucosal gel hydrophobicity and epithelial cell mitochondria and indeed other cellular processes, is increasingly being acknowledged after years of complacency.

Wallace, J.L. *et al.*, (2000) *Gastroenterology*, 119, 706-709
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Ohno, R. *et al.*, (2004) *J Pharmacol Exp Ther.*, 310, 821-827
Hotz-Behofsits, C. *et al.*, (2010). *Scand J Gastroenterol.*, 45, 822-877

S4

CENTRAL NEURAL PATHWAYS MEDIATING GASTROPROTECTION

K. Gyires

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4. Budapest, 1089. Hungary

It is well known that gastric acid and pepsin secretion are necessary for the development of peptic ulcer. However, in case of upper gastric ulcers the acid secretion is normal or decreased and about half of patients with duodenal ulcer have acid output in normal range. Consequently, defective mucosal resistance plays an important part not only in the development of gastric ulcer, but may have role also in the development of duodenal ulcer disease. It may be raised that pharmacologically driven increase of gastric mucosal resistance represents an important therapeutic weapon in gastric/duodenal ulcer, particularly in patients with normal or reduced acid secretion.

Gastric motility, gastric acid secretion and gastric mucosal defence may be regulated both centrally and peripherally. Peripheral factors and mechanism involved in gastric mucosal protection, such as mucus-bicarbonate-phospholipid barrier, surface epithelial cells, continuous cell renewal from mucosal progenitor cells, alkaline tide, mucosal microcirculation, sensory innervation of gastric mucosa and continuous generation of PGE₂ and PGI₂.

The role of central nervous system in maintaining gastric mucosal integrity has been well documented. Clinical observations showed increased gastrointestinal ulceration after intracranial lesion as well as in Parkinson disease (decrease of dopamine level), and decreased ulcer development in psychotic patients (increased dopaminergic tone) (Szabó *et al.*, 1982). Pharmacological evidence indicated that central administration of neuropeptides e.g. TRH, amylin, adrenomedullin, beta-endorphin, ghrelin, nociceptin, nocistatin (Tache *et al.*, 1994; review of Gyires, 2004; Brzozowski *et al.*, 2006; Zádori *et al.*, 2008) and non-neuropeptides (alpha-2adrenoceptor stimulants, cannabinoids) (Shujaa *et al.*, 2009) inhibited experimental ulcer formation. The site of action may be the dorsal vagal complex, paraventricular nuclei, locus coeruleus or hypothalamic nuclei. The centrally initiated gastroprotective effect may be conveyed to the periphery by vagal-dependent pathway as well as by the sympathetic nervous system. In the periphery CGRP, NO prostaglandins and as our recent findings showed somatostatin may mediate the centrally-initiated gastric mucosal protection.

Szabó S et al (1982) Lancet 2, 880P
Tache Y et al (1993) J Clin Gastroenterol 17 Suppl. 1, S58P
Gyires K (2004) Curr Top Med Chem 4(1), 63P
Zádori Z et al (2008) Peptides 12, 2257P
Shujaa N et al (2009) J Physiol Pharmacol 60 Suppl 7, 93P
Brzozowski T et al (2006) J Physiol Pharmacol 57 Suppl 6, 63P

S5

CRITICAL ASSESSMENT OF THERAPIES FOR SLE AND RELATED RHEUMATIC DISEASES

Ann Parke. University of Connecticut at St Francis Hospital and Medical School, Hartford, CT, USA

Aim To develop a critical assessment of therapies for Systemic Lupus Erythematosus (SLE) and related rheumatic diseases.

Methods A review of the recent literature pertaining to the management of SLE and related rheumatic diseases was conducted using several search engines including; PubMed, MD Consult and Google Scholar

Impressions Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by the production of non organ specific antibodies resulting in a small vessel vasculitis with the deposition of immunoglobulin and complement in affected vessels. The adaptive and innate immune systems both play a major role in the development of this disease that can result in significant morbidity and mortality especially when major organs are damaged. Cevera R (2009) addressing the epidemiology of SLE, using a European Cohort, determined that the survival rates of patients with SLE is 93% at 10 years compared to less than 50% surviving 5 yrs in 1950. As this is a disease of young women these rates are still unacceptable but this improvement is due to a better recognition of and an earlier detection of the disease as well as better antibiotics, improved antihypertensives and an expanded use of the 4 aminoquinilone drugs, in particular hydroxychloroquine. A prospective study addressing the effects of antimalarials demonstrated that hydroxychloroquine increased survival in SLE patients and protected against thrombosis (Ruiz-Irastorza, 2006), one of the factors known to contribute to an increase risk of mortality.

Phospholipid antibodies (aPL) are known to promote clinical thrombotic events, both arterial and venous and sometimes they are recurrent. Some, but not all of these patients also make criteria for SLE but the aPL syndrome is now considered to be part of the spectrum of autoimmune rheumatic diseases and aggressive and in some cases lifelong anticoagulation has been the treatment of choice. RHM Derksen and PG de Groot (2010) recently made the plea for “evidence based treatment of thrombotic aPL syndrome” claiming the data used to develop the current treatment recommendations are inaccurate and incomplete.

The complete pathogenesis of SLE is still not fully understood. More than 30 risk alleles have now been identified and the cross reactivity of antibodies, considered to be quite specific for SLE, with viral proteins carrying similar peptide sequences as well as an increased Epstein Barr (EB) viral load and elevated levels of interferon alpha (IFN- α) that correlate with disease activity and severity have helped to fuel the development of new treatments directed against IFN- α . Several different monoclonal antibodies targeting IFN- α are currently in clinical trials but initial results appear to be promising (Yao 2009). Infection remains a major concern as is the case with many of these targeted therapies including tocilizumab a monoclonal antibody

directed against IL-6 that has also demonstrated clinical improvement in SLE patients (Illei G 2010). Other agents targeting TNF α and INF- γ are also in clinical trials.

Elevated levels of B-lymphocyte stimulator (BLyS), a cytokine essential for the survival of B lymphocytes correlate with disease activity in patients with SLE. Recently Navarro (2011) confirmed that phase III, randomized placebo controlled blinded studies of belimumab, a monoclonal antibody that targets BLyS, demonstrated a clinical improvement when given with standard of care with no increase in rate of adverse events compared to placebo.

The FDA has now approved belimumab for use in patients with SLE. Other anti Bly-S and anti APRIL agents are also in clinical trials. Mycophenolate mofetil, an inhibitor of both T and B cell proliferation, and rituximab, another anti B cell agent (anti-CD20) continue to be used off label for patients with severe uncontrolled organ and life threatening disease. Numerous other new agents are also in development including other anti-cytokine agents, quinoline derivatives and modifications of glucocorticoid drugs designed to minimize the toxicity of these very important agents that still remain the mainstay of treatment for SLE patients.

Cevera R et al (2009) *Lupus* 10: 869.

Derksen RHM, de Groot PG (2010) *Lupus*, 19: 470.

Illei G et al (2010) *Arth Rheum* 60: 542.

Navarro SV et al (2011) *Lancet* 377: 721.

Ruiz-Irastorza G et al (2006) *Lupus* 15: 577.

Yao Y et al (2009) *Arth Rheum* 60:1785.

S6

EVALUATION OF BIOLOGICS IN RHEUMATOID ARTHRITIS

Andrew J K Östör, Department of Rheumatology, University of Cambridge, Cambridge, UK

The treatment of rheumatoid arthritis (RA) has seen unprecedented change over the last decade. Previously considered a progressive multisystem autoimmune disorder leading to inexorable decline the goal of treatment is now complete disease remission. The previous ‘start low, go slow’ therapeutic pyramid has been resoundingly rejected in favour of early intensive intervention, in order to switch off the inflammatory process as comprehensively as possible. It is clear that inflammation is the enemy as highlighted by the Treat to Target initiative and recent NICE RA guidelines (Smolen et al., 2010; www.nice.org.uk).

The recent advances have been spear-headed by the introduction of biologic disease modifying anti-rheumatic drugs (DMARDs). These agents selectively target pro-inflammatory cytokines and cells responsible for the clinical manifestations of the disorder. Overall biologics, in combination with the pivotal traditional DMARD methotrexate, have lead to improvement in multiple aspects of RA including the radiographic damage, disability and quality of life. In addition there is evidence emerging for the benefit of biologics in abrogating endothelial dysfunction occurring as a consequence of unfettered inflammation, thus reducing cardio-vascular morbidity and mortality (Westlake et al., 2011).

Currently four classes of biologic agents are routinely available for the treatment of RA. These include the anti-TNF agents (infliximab, etanercept, adalimumab, certolizumab and golimumab), the B-cell depleting agent rituximab, the T-cell co-stimulatory molecule blocker abatacept and the IL-6 receptor antagonist tocilizumab. This allows the opportunity to trial a number of drugs in a patient who fail to respond to a particular agent. This has dramatically altered the prognosis of many patients with RA around the world however at present we do not know the optimal sequence of drugs. NICE have developed biologic recommendations although the hurdle to obtain biologics is high in the UK due to cost-effective considerations (www.nice.org.uk). The uptake has been greater in other developed regions such as Europe, Scandinavia and the US.

The future is looking very bright in this field. The focus at present is to identify biomarkers of response in order to individualise therapy as currently we employ a blunderbuss approach simply moving from one biologic to another depending upon response and tolerability. In addition orally administered small molecules such as JAK-STAT and Syk inhibitors, which alter intra-cellular inflammatory signalling, are being trialled in RA.

Overall a sea change has occurred in the management of RA resulting from suppression of inflammation by biologic agents. As the therapies become more sophisticated we may eventually individualise treatment leading to not only a disease free but also drug free state, a concept unthinkable even 10 years ago.

Smolen JS et al. Treating rheumatoid arthritis to target: recommendations of an international task force. *Ann Rheum Dis* 2010 ;69:631-7

www.nice.org.uk

Westlake SL et al Tumour necrosis factor antagonists and the risk of cardiovascular disease in patients with rheumatoid arthritis: a systematic literature review. *Rheumatology* 2011;50:518-31

S7

THE PLACE OF ANALGESICS; PRESENT AND FUTURE

¹Garry G. Graham, ²Michael J. Davies, ¹Richard O. Day, ³Anthoulla Mohamudally & ¹Kieran F. Scott.

¹Department of Clinical Pharmacology, St Vincent's Hospital, NSW 2010 and Department of Pharmacology, University of New South Wales, ²Heart Research Institute, Sydney and ³Territory Palliative Care, Royal Darwin Hospital, Australia.

The place in therapy and the future development of three classes of analgesics depends to a considerable extent on perceptions about their mechanism of action. The three classes are:

- i) the classical non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit both cyclooxygenase-1 and -2 (COX-1 and COX-2). This group includes the older classical NSAIDs such as indomethacin, naproxen and ibuprofen.
- ii) the selective COX-2 inhibitors, such as celecoxib.
- iii) peroxidase inhibitors, which inhibit prostaglandin synthesis through their oxidation by the peroxidase function and consequent inhibition of COX-1 and COX-2. Paracetamol is by far the most important member of this class although metamizole (dipyrone) may have a very similar action through its active metabolite, methylaminoantipyrine. The potent inhibition of prostaglandin synthesis by paracetamol is seen in intact cells when

the peroxide tone is low and its efficacy is very similar to that the COX-2 inhibitors. It is still widely stated that paracetamol selectively blocks a splice variant of COX-1 which has been termed “COX-3”. However, selectivity of paracetamol for “COX-3” in intact cells has not been shown conclusively *in vitro* and, furthermore, an enzymatically active “COX-3” has not been demonstrated in humans (Graham and Scott 2005).

Unlike the non-selective NSAIDs and the selective COX-2 inhibitors, paracetamol does not have anti-inflammatory activity in rheumatoid arthritis although therapeutic doses have anti-inflammatory activity under conditions of lesser inflammation, such as tissue swelling after oral surgery.

The non-selective NSAIDs, selective COX-2 inhibitors and paracetamol all have both central and peripheral effects. This is shown by the following data:

- i) All three groups have anti-nociceptive activity after both systemic and small doses administered by intrathecal injection; indicating a central effects. (Pelissier *et al.* 1996)
- ii) Although the data are sometimes inconsistent, the antinociceptive effects of paracetamol and NSAIDs are often reversed by antagonists of central neurotransmitters, including serotonin (Pelissier *et al.* 1996), opiates (Franca *et al.* 2006), endogenous cannabinoids (Fowler 2004), substance P and the central noradrenergic agonist, clonidine. Again, these data indicate central effects of both classes. Antagonism by the analgesic effect of paracetamol by serotonin antagonists has been demonstrated in man.
- iii) Local injection of paracetamol decreases prostaglandin E₂ release in surgical sites following removal of molar teeth. (Lee *et al.* 2007) Paracetamol also inhibits the synthesis of prostaglandins by a variety of peripheral cells (showing peripheral effects). (Graham & Scott 2005)

Overall, the analgesic effect of paracetamol, like that of the NSAIDs and the selective COX-2 inhibitors, appears to be based on inhibition of prostaglandin synthesis that is occurring both centrally and peripherally. Despite the parallel gross actions of paracetamol and the non-selective NSAIDs, there are favourable interactions between these drugs, with both additive and synergistic effects, in experimental and clinical pain. Similar favourable interactions may exist between the selective COX-2 inhibitors and paracetamol.

The major pharmacological difference between paracetamol and the NSAIDs is that paracetamol is a substrate for myeloperoxidase and, consequently, inhibits the production of hypochlorous acid, hypobromous acid and hypothiocyanous acid. (Koelsch *et al.* 2010) These oxidants have been implicated in the development of atherosclerosis and, consequently, paracetamol may be useful in the treatment of atherosclerosis. Several plant polyphenols are also inhibitors of myeloperoxidase and their use in atherosclerosis should be investigated further.

The hepatotoxicity of overdoses of paracetamol is of great concern but the clinical association of the overdoses varies in different countries. In USA, 48% reported unintentional overdosage, with 44% overdoses taken with suicidal intent. By contrast, the unintentional and intentional overdoses accounted for 17% and 75% of total overdoses in UK. The reasons for the higher rate of unintentional overdose in USA are contentious but almost certainly include the very high prescription rates of combination tablets of paracetamol (presently 500 mg, but to be reduced to 325 mg) and hydrocodone and oxycodone (10 mg).

It is well known that the hepatotoxicity of paracetamol is due to its oxidative metabolism by cytochrome P450 enzymes, particularly cytochrome P450 2E1, to a thiol reactive compound. As the therapeutic effects of paracetamol are due to its metabolism by peroxidase function of COX-1 and COX-2 and, possibly by myeloperoxidase, selective activity of novel compounds towards these peroxidases could well be therapeutically useful.

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S8

CHONDROITIN SULFATE AS A MODEL FOR NATURAL THERAPY OF OSTEOARTHRITIC DISEASE

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Chondroitin sulfate (CS) is a biomolecule extracted and purified from several tissues and organs possessing important biological and pharmacological properties, such as anti-inflammatory and chondroprotective activities. Actually, CS is recommended by EULAR as a SYSDOAA (Symptomatic Slow Acting Drug for OA) drug in Europe in the treatment of knee, hip and hand OA. Furthermore, recent clinical trials demonstrated its possible structure-modifying effects.

Along with clinical trials, several animal models have been used to evaluate CS treatment in an effort to understand the molecular basis of its efficacy. For example, orally administered CS in type II collagen-induced arthritis mice significantly inhibited hind paw edema, synovitis and destruction of the articular cartilage (Omata *et al.*, 2000). Quite comparable results were observed in the same animal model by administration of LMW-CS (Cho *et al.*, 2004) and CS from *Raja* Cartilage (Jin *et al.*, 2007). Oral intake of CS inhibited the specific IgE production and antigen-induced anaphylactic response by up-regulating regulatory T-cell differentiation, followed by down-regulating the Th2 response (Sakai *et al.*, 2006). In a rabbit model of articular cartilage injury, Condrosulf® was found to have a protective effect on the damaged cartilage (Uebelhart *et al.*, 1998). Additionally, CS administration reduced the concentration of the proinflammatory molecules and the nuclear translocation of nuclear factor-kB in a rabbit model of atherosclerosis aggravated by chronic arthritis (Herrero-Beaumont *et al.*, 2008). Finally, highly pure CS was capable to significantly reduce the severity of arthritis along with the oxidative stress, a consequence of chronic inflammatory processes occurring in adjuvant arthritis (Manuscript in preparation). Overall, CS proved to have a beneficial effect in slowing down arthritis development and in reducing disease markers supporting the beneficial activity in humans as drug.

These results in animals and humans models describe the different mechanisms behind the anti-inflammatory and chondroprotective actions of actions demonstrated in a number of clinical trials. Due to its properties and safety, CS represents a model of natural drug for the treatment of OA disease.

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S9

NOVEL AGENTS FOR CONTROLLING INFLAMMATION AND CANCER

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The control of the diversity of inflammatory reactions that are present in arthritis and cancer has presented challenges to the medicinal chemist and pharmacologist. The key issues have been in target identification. Historically, prostaglandins (PGs) were considered the main target in both conditions but as further insight has been obtained from molecular and cell biology of these states then new receptors or enzymes have proven attractive for specific targeting of new small molecules. Over the past 2-3 decades new non-steroidal anti-inflammatory drugs (NSAIDs; e.g. coxibs, COX-LOX inhibitors, NO-NSAIDs) were developed with the prospect of being safer to the gastro-intestinal tract than traditional NSAIDs (e.g. diclofenac, naproxen). These have proven to have limited success. In cancer, NSAIDs (traditional and newer drugs) have proven to be promising chemopreventive agents in multiple cancer targets in experimental, epidemiological and clinical settings. Notably, COX-1/2 inhibitors, compounds with other mechanisms such as modulation of lipoxygenase (5-, 8-, 12- 15-LOX-1, 15-LOX-2), PHE₂-synthase inhibition (e.g. mPHES-1), inhibition of COX product (prostaglandin) receptors, and NO-releasing NSAIDs have shown promise on the basis of improved efficacy and/or reduced toxicity in arthritis as well as in cancers.

Prostaglandins as targets are of particular interest involved since they are involved in the growth of many cancers. They are major foci in colon cancer prevention in patients with familial adenomatous polyposis and some pre-malignant lesions, Barrett's dysplasia, oesophageal cancer, bronchial metaplasia, basal cell nevi actinic keratoses and even glioblastoma multiforme. However, some significant PG-independent mechanisms of NSAIDs have been identified which are of importance as mechanisms that may control these conditions (e.g. PPAR δ).

Lipoxygenase modulation to reverse carcinogenesis is a complex mechanism as well. Recent studies revealed procarcinogenic lipoxygenases (5-, 8-, and 12-LOX) and anticarcinogenic 15-LOX-1 and possibly -2. All together, these data led to propose that the various COX/LOX pathways exist in a dynamic balance that may shift during carcinogenesis toward 5-, 8-, 12-LOX and COX-2. A novel approach for cancer chemoprevention would therefore involve multitarget COX/LOX modulators shifting the balance from procarcinogenic to anticarcinogenic metabolism of polyunsaturated fatty acids.

Recent research is going further beyond the target receptors or enzymes involved of eicosanoid metabolism. Among these are agents focusing sphingolipid signalling (ceramide, sphingosine 1-phosphate, ceramidase, sphingosine kinase-1, sphingomyelinase, ceramide synthases). Moreover, protein kinases and other regulators of signal transduction (e.g. NF κ B, p38 MAPK, PI3Ks, JAK3) are attracting much interest. To date few agents have proven effective in experimental models or early clinical trials and there have been many disappointments. Amongst out strategies we have to developed dibenzosuberones targeting

p38 and protein kinases (Laufer et al, 2006). With some of these we optimized them down to single digit nanomolar IC50s against p38 which have excellent selectivity profiles against other protein kinases.

A major issue concerning the chronic use of established NSAIDs as well as newer anti-inflammatory agents for long-term prophylactic use is the essential requirement for low toxicity, which now this has to be a major focus.

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S10

IAPs IN AUTOIMMUNE DISORDERS

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An emerging body of evidence/literature highlights the potential role of cIAPs in immune disease: Apoptosis pathways appear defective in active RA synovial tissue; IAP overexpression/induction causes apoptotic resistance and modulation of differentiation of several types of pathogenic immune cells (macrophages, FLS)¹. There is a correlation of IAP overexpression in tissue from patients with status of active autoimmune diseases (RA, MS) and successful DMARD treatment of RA patients may restore apoptosis in synovial tissue by reducing apoptotic pathway inhibition, including downregulation of IAPs². Two additional mechanisms may also contribute to the effectiveness of IAP inhibition in inflammation /RA: Interference with RANKL signalling by IAP inhibitors may impact osteoclastogenesis and a down regulation of innate immune responsiveness to certain TLR and NOD ligands. We have identified several classes of compounds which inhibit XIAP and which cause profound systemic ablation of cIAPs1 and 2. These compounds are ideal candidates to test the hypothesis that IAP modulation may have therapeutic effects in disease models of autoimmune disorders. Nevertheless, the possibility that immune activation may also be a consequence of IAP inhibition is of concern. The identification of compounds with the desired pharmacodynamic, pharmacokinetic and tolerability profile will be dependent on a thorough investigation of the therapeutic index of this class of compounds.

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S11

CONDITIONAL PHARMACOLOGY, PHARMACOGENOMICS, EPIGENETICS: ISSUES FOR DRUG DEVELOPMENT AND USE.

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Almost all currently marketed drugs were tested and approved on the basis of clinical studies in middle aged patients mostly of European ancestry and with a single dominant disease, typically with excellent access to medical care. But these drugs are not always useful and safe for individuals. To understand individual responsiveness, and to tailor drug development for individuals, we need to consider conditional pharmacology, pharmacogenomics and epigenetics.

Conditional pharmacology (Whitehouse 1991) posits that the condition (disease or stress state) influences the therapeutic response and, implicitly, the side (adverse) effects of drugs. Extending this concept, one might thus ask to what extent do multiple diseases, as may be found in developing countries and in the elderly, affect therapeutic outcomes by altering the safety and/or efficacy of drugs? Clinical trials cannot be reasonably conducted to evaluate all possible conditions, so population pharmacokinetics generally have been used to provide some answers to conditional pharmacology issues in populations, such as use in children, in the elderly and in different ethnic groups, but still does not address individual patient responses.

Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs (pharmgkb.org). While genomics can help better define the individuals who might benefit most and exclude those who will either not benefit and/or suffer toxicity from pharmacokinetics of existing drugs, can it really be used in a predictive manner to develop new chemical entities? And what if the identified subpopulation is too small to be economically feasible to develop a treatment? Will pharmacogenomics create subsets of "rare diseases" within existing disease categories? Can it be used to deny treatment or as an excuse for treatment failure?

Epigenetics is the study of changes in gene activity that do not involve alterations to the genetic code but still get passed down to at least one successive generation. It is through epigenetics that environmental factors like diet, stress and prenatal nutrition and chemical exposure/pollution can make an imprint on phenotype that is passed from one generation to the next. Since even identical twins diverge in disease incidence as a function of environmental factors (Wong et al. 2005), epigenetics affects present as well as future generations. Might epigenetics, in addition to standards of care, be a factor in assessing clinical trial data from patient populations with distinct dietary or environmental experience?

Considering all the factors that may influence drug efficacy and safety, such as age, disease(s), drug-drug interactions, epigenetics, genomics etc., as well as, the costs of drug development and the politics of healthcare, how realistic are promises of personalized medicine?

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FUTURE THERAPY FOR GOUT

G.Nuki.

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Gout became a paradigm for understanding, treating and preventing a chronic rheumatic disease following the introduction of the xanthine oxidase inhibitor allopurinol as an effective urate-lowering therapy (ULT) nearly 50 years ago (Rundles et al., 1963). After an early increase in the frequency of flares that follows initiation of any ULT, lowering serum urate below its solubility threshold was followed by resolution of tophi and a progressive decrease in the number of attacks of acute gouty arthritis. Yet, despite the availability of effective uricostatic and uricosuric ULT, there has been a worldwide increase in the incidence and prevalence of gout over the last 30 years (Roddy and Doherty 2010) in association with rising levels of serum urate (SUA), cardiovascular co-morbidity (Annemans et al., 2008) and a six-fold increase in the prevalence of the metabolic syndrome (Ford et al., 2002). Gout has become the commonest type of inflammatory arthritis in men and an increasingly frequent cause of inflammatory joint disease in women. An overall prevalence of 1.4% in the UK and Germany, rises to 3% in women and over 7% in men over the age of 75 years (Mikuls et al., 2005; Annemans et al., 2008). A recent Europe-wide study of 1380 gout patients treated with ULT in specialist clinics or in General Practice showed that 66% had a serum urate (SUA) higher than the EULAR recommended target of $360\mu\text{mol/L}$, 32% had tophi and more than half had clinical evidence of chronic inflammatory joint disease (Nuki et al., 2011). Clearly there is a substantial subset of chronic gout patients whose SUA and clinical manifestations of gout are not controlled despite treatment with existing ULTs. Much of this failure to control what is a potentially curable disease could be rectified by lifestyle modification and careful 'treatment to target' with long established uricostatic and uricosuric ULTs. However, new options are becoming available for the minority of patients who do not respond adequately or who cannot tolerate existing ULTs, for the first time in half a century. In the future these are likely to include monotherapy with the non-purine xanthine oxidase inhibitor febuxostat (Jansen et al., 2010), dual uricostatic therapy with allopurinol and a purine nucleoside phosphorylase inhibitor (Hollister et al., 2011), combined uricostatic and uricosuric therapy with allopurinol and a URAT-1 inhibitor, Lesinurad (Perez-Ruiz et al., 2011), or biologic therapy with the pegylated recombinant uricolytic uricase, pegloticase, for patients with severe, refractory and tophaceous gout (Sundy et al., 2008).

The efficacy and safety of current options for treatment and prophylaxis of acute gout with colchicine, non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids is also sub-optimal. Macrophage production of interleukin- 1β (IL- 1β) in response to urate crystals was demonstrated more than 20 years ago (Di Giovine et al., 1987). Recent advances in understanding the role of the NALP3 inflammasome, caspase-1 activation, cleavage of pro-IL- 1β and secretion of active IL- 1β in uric acid crystal- induced inflammation (Martinon et al., 2006), and the possible role of free fatty acids as second signals (Joosten et al., 2010), has highlighted the importance of IL- 1β as a major mediator, and likely target, for controlling, and preventing inflammation in acute gout. Options that are currently being explored include the IL- 1β receptor antagonist, anakinra (So et al., 2007), the IL-1 receptor fusion protein riloncept (Schumacher et al., 2009) and the human monoclonal anti-IL 1β antibody Canakinumab (So et al 2010).

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S13

PHOSPHOLIPID COMPLEXES OF NSAIDS AS GI SAFER DRUGS.

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Our lab has focused upon the elucidation of the mechanism by which nonsteroidal anti-inflammatory drugs (NSAIDs) induces surface injury to the GI mucosa. In this body of work, we have demonstrated by contact angle analysis that the surface of the upper GI tract is endowed with unique surface hydrophobic properties, which protect the tissue from luminal acid that is mostly attributable to the presence of phosphatidylcholine (PC) and related phospholipid surfactants within and coating the mucus gel layer. Furthermore, we have shown that luminal NSAIDs have the capacity to rapidly attenuate the stomach's non-wettable surface property due to their ability to non-covalently associate with PC (Lichtenberger *et al.* 1995). In addition, we have subsequently demonstrated that NSAIDs can also compromise the surface membrane of GI epithelial cells, resulting in the formation of unstable pores to promote the back-diffusion of luminal acid (Lichtenberger *et al.*, 2006). This surface injurious action of NSAIDs can be further exacerbated in the presence of bile acids, as recent evidence, based upon Nuclear Magnetic Resonance (NMR) imaging, Molecular Dynamic (MD) simulation and cell culture studies suggests that these two classes of amphipathic molecules have the capacity to aggregate into toxic mixed micelles to disrupt the brush border membrane. We have also determined that pre-association of PC with NSAIDs using a simple method of formulation can provide marked protection against the surface injurious action of NSAIDs in a number of rodent model systems, including the model where aspirin and celecoxib are used in combination (Lichtenberger *et al.*, 2007, 2009). Recently published clinical trials have also demonstrated the PC-conjugate of ibuprofen and aspirin significantly provide protection against NSAID-induced endoscopically observable gastroduodenal erosions/ulcers in "at risk" subjects >50 years of age (Lanza *et al.*, 2008, Cryer *et al.* 2011). In summary PC-complexed NSAIDs represent a novel class of

NSAIDs that provide significant GI protection against NSAIDs (aspirin and ibuprofen) that primarily cause ulceration/bleeding due to surface injury. This new class of PC-NSAIDs may also have clear advantages over other strategies currently available or being developed to reduce NSAID-induced GI injury by maintaining the stomach's normal acidic environment, with minimal risk of side-effects.

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S14

UNDERSTANDING HOW TO MAKE GLUCOCORTICOIDS GI SAFE

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It is enough known that Edward Kendall, Tadeus Reichstein and Philip Hench were awarded the Nobel Prize in Physiology or Medicine in 1950 “for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects”, for the first demonstration of beneficial actions of “the hormones of the adrenal cortex” in patients with chronic rheumatic arthritis. Yet few in the fields of biomedical sciences recall that the discovery of anti-inflammatory action of glucocorticoids & the pro-inflammatory properties of mineralocorticoids were first demonstrated in animal models about 10 years earlier & the name of these steroids also originate from Hans Selye who published these very original studies in the best journals of all time, e.g., *Science* 1941; *Nature* 1943; *Am. J. Physiol.* 1943; *Endocrinology* 1942; *Can. Med. Assoc. J.* 1942; *Lancet* 1943, 1946; *JAMA* 1944 (Szabo & Tache, 2007).

After the demonstration of beneficial glucocorticoids' actions in patients with chronic rheumatic arthritis, suddenly, a vast new field of medical research was opened. However, from the early trials Hench et al were also well aware of the many adverse effects of cortisone therapy. These adverse effects have been repeatedly confirmed by extended clinical experiences over the past 60 years (Whitehouse, 2011). There is a long list of undesirable side effects associated with glucocorticoid therapy and gastrointestinal (GI) side effects are one of the serious complications.

How can we make glucocorticoids GI safe? One of the ways of solving this problem may very well be found in a proper understanding of glucocorticoids' contribution to the physiological processes that take place in the GI mucosa and through further explanation of the question of how physiological gastroprotective action can turn into a pathological proulcerogenic action.

It is known that basal glucocorticoid production contribute to the maintenance of the gastric mucosal integrity. According to our data an acute stress-induced increase of glucocorticoids also has a gastroprotective action against stress-induced gastric injury (Filaretova *et al.*, 1998, 2008), but is not ulcerogenic, as it has generally been considered for some decades. Beneficial action of high levels of endogenous glucocorticoids released during acute stress on the stomach is opposite to the harmful actions of exogenous glucocorticoids at pharmacological doses used as a hormonal therapy. NSAIDs, similar to stress, induce an increase in glucocorticoid production that in turn helps the gastric mucosa to resist the harmful actions of these stimuli (Filaretova *et al.*, 2002). These results suggest that the increased risk of adverse gastric reactions should be considered when NSAIDs are used in

patients with impaired glucocorticoid production. Glucocorticoids exert gastroprotective actions in co-operation with PGs, NO and capsaicin-sensitive sensory neurons: their compensatory gastroprotective action is observed when the protective mechanism provided by either of these factors is impaired (Filaretova *et al.*, 2007). Gastroprotective effects of glucocorticoids may be mediated by multiple actions, including maintenance of gastric mucosal blood flow, mucus production, and attenuation of enhanced gastric motility and microvascular permeability (Filaretova *et al.*, 2002, 2007). The contribution of glucocorticoids to gastroprotection is tightly related with their contribution to general body homeostasis. Glucocorticoids released during activation of the hypothalamic-pituitary-adrenocortical (HPA) axis may contribute to protection of the gastric mucosa by maintaining general body homeostasis, including glucose levels and systemic blood pressure, which could be a basis for their beneficial influence on gastric mucosal integrity. These findings further support idea that gastroprotective action of glucocorticoids is an essential element of their general adaptive action. The results obtained suggest that glucocorticoids released during acute activation of the HPA axis are naturally occurring protective factors that play an important role in maintenance of the gastric mucosal integrity. In physiological conditions, even in acute stress situations, glucocorticoids have an adaptive effect on the stomach and, therefore, are gastroprotective, while in some situations their action on the gastric mucosa can turn into a pathological proulcerogenic action. Because the maintenance of glucose homeostasis by glucocorticoids could be fundamental to their gastroprotective action (Filaretova *et al.*, 2002), it was reasonable to assume that glucocorticoid-induced disturbance of glucose regulation, observed in clinical and experimental situations, may contribute to ulcerogenic action of glucocorticoids on the gastric mucosa. The data obtained so far suggest that short-lasting maintenance of blood glucose levels may be responsible for the gastroprotective action of glucocorticoids, while glucocorticoid-induced long-lasting maintenance of blood glucose levels accompanied with the signs of their catabolic effect and glucocorticoid-induced corticosterone deficiency may be responsible, at least partly, for the transformation of gastroprotective action of glucocorticoids to their proulcerogenic effect in rats. Our results allow us to speculate that glucocorticoid-induced disturbance of carbohydrate regulation, which needs time for developing, contributes to appearance of ulcer symptoms after long-lasting hormonal therapy. It means that control of glucose regulation and its correction in case of need may be considered as useful approach minimizing GI side effects of glucocorticoid therapy.

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ABSTRACTS POSTERS

P1

GALECTIN-9 IN INFLAMMATION: SWEET OR SOUR?

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Galectins are structurally related proteins characterised by their carbohydrate recognition domains and an affinity for β -galactosides. Galectin-9 (Gal-9) was initially characterized as a potent eosinophil chemoattractant (Matsumoto et al., 1998) but has subsequently been found to have profound immunosuppressive effects in models of collagen-induced arthritis (Seki et al., 2007) and carageenan paw oedema (Iqbal et al., 2011). Its effects on neutrophil recruitment and behaviour during inflammation have not been thoroughly investigated. The objectives of the present study were therefore to investigate whether Gal-9 modulates neutrophil recruitment during the acute inflammatory response.

The effect of Gal-9 on neutrophil recruitment was investigated using a static transmigration assay; transmigration of neutrophils through a confluent endothelial monolayer towards Gal-9 (0.1-300nM) and IL-8 (30ng/ml) was assessed in a transwell system. To further elucidate whether Gal-9 affects a specific step of the leukocyte recruitment cascade flow chamber assays were also performed. Freshly isolated neutrophils were pre-incubated with Gal-9 (3-30nM) and perfused over endothelial monolayers and the number of interacting neutrophils quantified. Adhesion molecule expression, annexin V binding and ERK phosphorylation was also assessed.

Gal-9 (10nM) significantly promoted neutrophil transmigration in the static assay (203.18 ± 3.29) compared to vehicle control (72.25 ± 3.29 , $p < 0.001$ one-way anova, $n=5$). The flow chamber experiments identified a specific effect of Gal-9 on neutrophil transmigration; pre-treatment of neutrophils with Gal-9 resulted in a significant increase in the number of transmigrated neutrophils (31.5 ± 4.32) compared to control (12.17 ± 3.46 , $p < 0.05$ one-way anova, $n=5$). A concomitant reduction in neutrophil rolling was also observed (10nM Gal-9 10.33 ± 3.92 vs Con 62.14 ± 17.1 , $p < 0.05$, one-way anova, $n=5$). Pre-treatment of Gal-9 was found to induce Annexin V binding in the absence of other activating stimuli and induced ERK phosphorylation.

In conclusion, these data indicate a role for Gal-9 in neutrophil recruitment during acute inflammation with a specific effect on transmigration indicating a novel role for this protein during acute inflammation.

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P2

THE MUSKULOSKELETAL PROBLEMS OF THE SIMILUAN ICEMAN: RELATIONSHIP TO “MEDICINAL” TATTOOS.

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In 1991 a human male deceased specimen was found frozen in a glacier pool in the Italian Alps in north west Italy, and is now carefully preserved in the South Tyrol Museum of Archeology, in Bolzano, Italy. The bodily tissues of the 5300 year old male were very well preserved despite damage related to freezing, and glacial movement. Articles of clothing, tools, weapons and other devices were also very well preserved. The male specimen is commonly colloquially referred to as the Iceman or Otzi. The clothing, tools, weapons and other artifacts have been studied in detail. In addition, scientific experts of numerous disciplines have studied the body tissues, the intestinal contents, and bodily contaminants, and where relevant have subjected these to investigation by gross anatomy, pathology, histology, bacteriology, biochemistry, and DNA structure. There exists an excellent knowledge base on the Iceman's point of origin, habitat, activities, work habits, and type of food intake (Fecklinger 2005). Clinical examination and imaging investigations have also shown that the Iceman had experienced possible illnesses in his lifetime and had identifiable areas of arthritis and musculoskeletal injury (Murphy et al 2003).

We have performed a clinical examination of the Iceman, and with permission, have studied the available photographs and imaging pertaining to the musculoskeletal findings of the Iceman. The skin of the Iceman has numerous linear carbon tattoos, which are not of a decorative type. These have been presumed to possibly be (“medicinal”) tattoos administered for therapeutic reasons, (Fecklinger 2005) and may have been administered for the treatment of pain. Spinal imaging has provided clues as to possible sites of pain and hence sites for administration of the “medicinal” tattoos (Murphy et al 2003).

We have noted that there are body areas of the Iceman, in which imaging has demonstrated arthritis and other forms of long-term musculoskeletal damage, but which do not have adjacent or corresponding “medicinal” tattoos.

We contend that several of the “medicinal” tattoos correspond directly to sites of spinal area pain plus referred neurosensory pain (Murtagh and Kean 2008), and to sites of peripheral joint pain, which would have been experienced by the Iceman.

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THE GREENING OF HEALTHCARE: FABRICS USED IN HEALTH CARE FACILITIES

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In all healthcare settings, the local environment can have an influence on the health of all personnel present in that healthcare environment, including physicians, surgeons, laboratory researchers, nurses, administrators, etc., and of course patients and their visitors or companions. There are many factors which influence the healthcare environment and issues such as commonly observed issues such as air quality, temperature control and infection control are in the main reasonably well addressed. However it has only been in the last 18 years or so that there has been a structured attempt to control the quality of healthcare facility construction and the contents of that construction, including fabrics.

The “greening” of healthcare textiles is thus a topic of great importance due to the large numbers of chemicals used in the production and maintenance of fabrics and hence exposure of these fabric substances and by-products to both patients and all healthcare workers, in the form of dermal contact, inhalation, and ingestion.

The Leadership in Energy and Environmental Design (LEED) is a comprehensive internationally recognized standard for certification and construction of green buildings (Canada Green Building Council, 2004). There are general principles of standards for green construction, and for construction materials, that have been established by LEED. The ones used to provide a framework for selecting textiles include the following 5 criteria:- Local Source Availability; Durability; Sustainability; Recyclability; Surface Finishes & Cleanability.

There are several certification programs that are pertinent to the textile industry. Labels on healthcare and other commercial fabrics display applicable certifications of the chemicals which are tested and why these chemicals are of concern. The exposure risks, control of use, and thus certification for use of these chemicals in fabric production for healthcare settings, needs strict governance as to who provides that certification. While certification can be provided by governments and by industry, we contend the third party certification is the optimum route to provide unbiased certification information on chemical toxicity and exposure risks. These third party organizations include LEED, McDonough Braungart Design Chemistry, Öeko-tex, Greenguard Environmental Institute, Scientific Certification Systems, the Global Organic Textile Standard (GOTS), and the American Society of Testing and Materials.

Among the most commonly used chemicals in the manufacture and care of fabric used in healthcare are: Formaldehyde; Volatile Organic Compounds; Phthalates; Di(2-ethylhexyl)phthalate; Quaternary Ammonium Compounds; Vinyl Chloride; Polyurethane; Toluene diisocyanate; Antimony trioxide; Perfluorooctanoic acid; Polybrominated diphenyl ethers; Decabrominated diphenyl ether; and Triclosan. These have a documented range of toxicity, which include carcinogenicity, oestrogenicity, contact dermatitis; bronchoconstriction, and thyroid toxicity as identified by U.S. Center for Disease Control, Environment Canada’s Canadian Environmental Protection Agency, U.S. Environmental Protection Agency, the World Health Organization’s International Agency for Research on Cancer, and the International Programme on Chemical Safety Poisons Information Monograph (International Agency for Research on Cancer 2006).

Application of the LEED principles for selecting textiles namely the 5 criteria:- Local Source Availability; Durability; Sustainability; Recyclability; Surface Finishes & Cleanability, has provided an avenue to introduce and reintroduce potentially safer fabrics and textile products. These can be produced from familiar materials such as cotton, and hemp, and can utilize the bio-based polymers from corn, silicone, wood pulp, bamboo and soybean, among many other more natural products that will provide a safer thus “greener” healthcare environment (Greenpeace 2010).

In summary, healthcare textiles in current use may pose a serious risk to the health and safety of patients and all healthcare workers, when they are unknowingly exposed to the chemicals of concern. Many studies have been done on acute or chronic exposure of large doses of these chemicals and toxicity profiles have been identified. However it is difficult to assess the risk of exposure of these chemicals at low doses, over a long period of time, or consider the risk on patients who are already immuno-compromised. Governments appear to side with scientifically ‘proven’ hazards rather than risk the liability of banning a product that is only potentially harmful. Their approach is to work with manufacturers for voluntary discontinuation over a period of time. Environmental groups appear to be at the other extreme of demanding immediate bans. The middle ground suggests the advisability of selecting textiles that have third party certifications applicable to textiles. Decisions based on the criteria of sourcing locally, selecting durability, using credible standards of performance, selecting sustainable natural fibres, recycled or recyclable synthetic fibres, selecting minimal topical finishes, and selecting fabrics with ease of cleanability are advised for the greening of healthcare fabrics, and hence improving the quality of the healthcare environment..

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P4

OBESITY AND KNEE OSTEOARTHRITIS

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Obesity and Knee Osteoarthritis

The association between obesity and knee osteoarthritis, and specifically the role of obesity as a risk factor for knee osteoarthritis has been well documented.

A systematic review and meta-analysis by Blagojevic and colleagues (2010) examined 36 papers reporting on BMI and found that all studies demonstrated obesity and being overweight to be risk factors for knee osteoarthritis. Blagojevic and colleagues reported the

effect size for obesity as a risk factor for knee OA to be $I^2=97\%$, and the random effects pooled odds ratio for obesity compared to normal weight was 2.63 (2.28, 3.05).

A large, population-based prospective study with a follow-up of 22 years found that BMI was strongly associated with the risk of developing osteoarthritis of the knee, independent of covariates like age and gender (Toivanen et al 2010). Knee osteoarthritis was 7 times greater for people with $BMI \geq 30$ compared to the control of people with $BMI < 25$. These results were adjusted for covariates. The strength of this study and the results were the long follow up and population based prospective cohort study design.

A Norwegian population study of 1675 patients over 10 years surveyed patients to self-report knee osteoarthritis diagnosed by a physician and/or radiographically (Grotle et al 2008).

Height and body weight were also self-reported to calculate BMI. It was found that BMI was significantly associated with osteoarthritis of the knee, with odds ratio of 2.81, and 95% CI of 1.32-5.96. A dose-response relationship between obesity and knee osteoarthritis was found. Covariates and risk factors were adjusted for. Obesity was also found to be a statistically significant independent predictor for hand osteoarthritis, though this association did not exhibit a dose-response relationship. This is one of the few recent prospective studies to also report obesity as a risk factor for hand osteoarthritis.

BMI, waist circumference, waist-hip ratio, weight, and body fat percentage were monitored in a large, Swedish population based prospective cohort study that took place over 11 years (Lohmander et al 2009). Each of these measurements of 'overweight' was associated with incidence of knee osteoarthritis, with the strongest association being with BMI. This study was novel in that it considered different measures of overweight besides the standard BMI. The fact that all the different measures of overweight were significantly associated with incidence of knee osteoarthritis strengthens the link between the disease and obesity.

Sowers and colleagues (2008) performed a 4 year, longitudinal population based study following 541 women.¹⁴ Knee osteoarthritis was measured radiographically using the Kellgren-Lawrence scale ($KL \geq 2$), and joint space width was measured with electronic calipers. BMI, as well as body composition indicators fat mass, lean mass, skeletal muscle mass, and waist circumference were measured. It was found that skeletal muscle mass was a more consistent predictor of osteoarthritis, as well as significantly associated with the amount of joint space width. It was suggested that measures of body composition, such as skeletal muscle mass, could more consistently predict incident knee osteoarthritis compared with BMI, which does not take into account body composition and fat vs. lean mass.

Although obesity has been repeatedly confirmed as a risk factor for knee osteoarthritis, Niu and colleagues (2009) found that there was no overall association between obesity and the actual progression of knee OA.¹⁵ Among knees that were radiographically diagnosed with OA at baseline, BMI did not play a role in the progression of the disease. In particular, obesity did not affect progression of knee OA in knees with varus alignment, but there was some association with progression in knees with valgus and particularly with neutral alignment. However, there is some debate about studies examining risk factors for progression of knee osteoarthritis are methodologically sound, and so further investigation is needed.¹⁶

This review of the literature has examined recent publications investigating osteoarthritis of the knee, and any potential associations with obesity. Recent literature agrees that weight loss is beneficial, improving both pain and function in patients.

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Grotle, M., Hagen, K.B., Natvig, B., Dahl, F.A., Kvien, T.K. (2008). BMC Musculoskeletal Disorders; 9:132.

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Toivanen, A.T., Heliövaara, M., Impivaara, O., Arokoski, J. P.A., Knekt, P., Lauren, H., Kroger, H. (2010). *Rheumatology*;49:308-314.

P5

REBOUND RESPONSE INHIBITION AFTER WASHOUT OF THE P38 INHIBITOR SB203580. A NEW VIEW TOWARDS P38 INHIBITION?

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We have found that selective p38 MAPK inhibitors, SB203580 and ML3403, have inhibitory effects on adherent accessory cells such as macrophages when stimulated up to 120 hours after drug withdrawal, whereas no such effect is seen in lymphocytes (1). The relevance of this phenomenon, and how this occurs are matters for conjecture. The carry-over of effect could be as a result of cell response priming, or drug carry-over or sequestration. We have assessed the p38 MAPK signalling response after drug priming and washout, as well as Erk and ATF-2. In addition, drug retention was assessed, and correlated to intracellular MK2 phosphorylation.

As previously described (Malik et al, 2011), human monocytic U936 cells were differentiated to the macrophage phenotype by incubation with 80nM PMA for 48hr (U937MØ). After trypsinization, the cells were in 12 well plates at 5×10^5 /well. After overnight serum starvation, the cells were incubated with SB203580 for 2 hours before being washed 4 times and rested for various time-points. Cells were then stimulated with 100ng/ml LPS for 30 mins and supernatants were collected for TNF- α measurement by ELISA and whole cell lysates were prepared for western analysis of downstream targets of p38, transcription factors ATF-2 and MK-2. For drug washout, the U937MØ were prepared at 1×10^6 cells per well and incubated with 1 μ M SB203580 for 2 hours. They were then washed as above, and suspended in 100 μ L PBS prior to freezing and subsequent analysis by HPLC-mass spectrometry.

LPS stimulated TNF- α production by PMA-U937 cells was completely inhibited by SB203580 in a concentration related fashion (0.01-10 μ M). LPS stimulated a concentration-dependent increase in the phosphorylation of ATF-2 and MK-2. However, only phosphorylation of MK-2 was inhibited by SB203580 (1 μ M). Priming the cells for two hours with the drug, followed by washout, restored the ability of cell to respond to LPS with MK-2 phosphorylation by the macrophages 1 min and 2 hours after washout. However the inhibition of MK-2 phosphorylation by p38 MAPK was reasserted 4 hours after drug withdrawal and this inhibition maintained at 24 hours. pAKT remained unaffected

throughout, indicating messenger selectivity. LPS induced TNF synthesis followed this pattern with synthesis recovering immediately after drug washout and inhibition recurring at 2, 4 and 24 hours. Washing the U937MØ after two hours contact with 1µM SB203580 resulted in a first order decay from 76nM at 15 seconds, to 58nM at 1 hours, 40nM at 2, 26nM at 4 hours, decreasing to baseline of 18 and 19nM at 24 and 48 hours. Thus the recovery of p38 activity and TNF synthesis inhibition of p38 MAPK and TNF synthesis up to 2 hours after washout, and the inhibition of synthesis thereafter is inversely related to the cellular exposure to the inhibitor.

Conclusions.

Preconditioning of macrophages results in delayed inhibition of p38 MAPK and TNF synthesis days after drug withdrawal. Since this action does not apply to lymphocytes, pulse therapy of p38 inhibitors may provide a more selective treatment paradigm in TNF dependent inflammatory disease.

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Malik N et al (2011) *Ann Rheum Dis Annals Rheum Dis* 70(S2) A17.

P6

PRISTANE DOSE, ARTHRITIS SEVERITY, DISEASE PATTERN, AND HUMANE END POINTS AGREED BY LOCAL ETHICS COMMITTEE.

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Pristane-induced arthritis (PIA) is an MHC dependent polyarthritis with infiltrating activated CD4+ T cells, erosion of peripheral joints and greater susceptibility in females (Holmberg et al., 2006). Unlike collagen induced arthritis it has a chronic relapsing course rather than a single flare. A pilot dose-ranging study was carried out to determine the pristane dose that would induce relapsing arthritis without excessive adverse effects as defined by preset humane end-points (HEP) agreed by the named veterinary surgeon (JPM) and the Procedure Assessment Committee (Queen Mary College Local Ethics Committee).

Pristane (75ul, 100ul or 150ul, n=5) was administered under isoflourane anaesthesia, i.d. to the tail base to DA/OlaHsd rats. Controls received no injection. Rats were scored with a 44 point system recording one each for swollen or red digit, knuckles, midfoot and ankle/ wrist between day 5-25 every 2 days and between day 25-100 twice every week. Relapse triggered bidaily scoring. Paw swelling was measured with 0.02mm graduation callipers. Paws were frozen or fixed with paraformaldehyde. Adverse effects were monitored to Institutional ethical guidelines & approved by the UK Home Office. Rats were monitored daily for body mass (HEP: -25%), paw volume (HEP: 3x baseline); sledging; paw score (HEP: Max score for two paws on 4 consecutive days). Early culling before 4 days would be supported for spontaneous vocalisation, fur soiling, and any other event the observer warranted addressing.

For all pristane doses, inflammation followed an acute phase followed by a chronic relapsing phase. Front and hind paws responded with signs of disease by day 20 followed by decline finishing by day 40. Ankles persisted to day 70. Relapse occurred in front and hind digits

with some wrist involvement, whilst ankles and midpaws did not. Hind paw thickness only occurred in the acute phase, fully declined by day 40. In the chronic phase, front paws were often characterised by swollen knuckles and there was a high incidence of severe distortion of front digits. Pristane was best tolerated at 75ul and 100ul. 150uL induced severe disease such that 40% (2) of rats reached the paw score HEP from day 14-16 during the 'attack' phase and were culled. No rats reached the HEPs during the relapse phase. Ankylosis became severe during the chronic phase of the disease.

In PIA in DA/OlaHsd rats, chronic relapsing disease follows a course that differentiates between digits, wrists, ankles, and foot thickness. Doses of 75ul and 100ul elicited a less severe and more manageable disease. 150uL reached predetermined HEPs in 40% of rats during the acute phase. 75-100uL was found to be optimal, however severe ankylosis should be included as an HEP.

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Holmberg J et al (2006) J Immunol 176, 1172-1179.

P7

COMPARISON OF CYTOKINE EXPRESSION IN ACTIVE AND PASSIVE MURINE COLLAGEN ARTHRITIS AND A DIFFERENTIAL ACTION OF DEXAMETHASONE.

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Active (mCIA) and passive (antibody induced, aCIA) collagen arthritis in the mouse, are similarly inflammatory and erosive arthritides, but have different mechanisms of induction (reviewed Hetal et al, 2010). mCIA elicits an auto-antibody and cellular immune response on immunization with heterologous collagen in Freund's complete adjuvant. Disease coincides with autoimmune anti-collagen IgG antibody generation. aCIA is induced with an arthritogenic anti-mouse collagen antibody cocktail (Englert et al, 1986) and may thus be considered to comprise the latter phase of mCIA. The anti-inflammatory steroid dexamethasone is used as a standard inhibitor of both diseases. We aimed to investigate the cytokine profiles of both models, and the chronic action of dexamethasone.

mCIA was induced in dba-1 mice with bovine collagen in Freund's Complete Adjuvant (R Williams, Kennedy Institute), and boosted at day 21 with collagen in FIA. aCIA was induced with 2mg anti-CII antibodies (ArthritoMab™ MDBioscience) i.v. and boosted on day 3 with LPS according to the manufacturer's instructions. Dexamethasone was dosed at 0.1mg/kg.day, and stopped 24 hours prior to termination (mCIA, day 35; CIA, day 16). The hind paws were snap frozen, macerated by nitrogen gun, and the powder suspended in Extraction Regent (Invitrogen) / protease inhibitory cocktail (Sigma). Samples were homogenized using ceramic beads (Bertin), centrifuged and then cytokines assayed by Multiplex (Bioplex, Bio Rad Labs).

TNF, IL-6 and IL-17 were undetectable in all extracted joint tissue. IL1 α , IL1 β , IL2, IL4, IL10, IL12(p40), IL12(p70), and KC (murine IL8) did not differ between the two types of arthritis. The variances for IL12(p40) ($p < 0.01$) and IL2 ($p < 0.05$) were different with mCIA being the higher. Dexamethasone induced an apparent TH₂ bias with IL4 and IL10 being ($p < 0.05$) increased. IL12(p40) was reduced ($p < 0.05$), and whilst IL1 α and KC were reduced by 50% and IFN γ raised by 50%, these and all other cytokines were not altered significantly. In aCIA, dexamethasone did not induce IL4 or IL10, but induced a significant increase in IL1 α ($p < 0.01$), IL2 ($P < 0.05$), IL12(p70) and IFN γ .

The variance of mCIA was greater, reflecting disease 'take'. Dexamethasone would be expected to suppress the majority of cytokines. Here, the study was terminated 24 hours after the last dexamethasone dose. Thus in active mCIA, dexamethasone appears to switch the immune response to a Th2 dominated profile. Interestingly, IL12 (P40) was reduced, but not IL12 (P70). This could represent IL23, involved in CMI, or less likely, IL12 antagonist monomers or dimers. In aCIA pro-inflammatory cytokine rebound is seen. The inhibition of active auto-immune processes with chronic steroid therapy could induce a Th₁/Th₂ switch that is maintained after acute withdrawal without rebound. This could have relevance to pulse therapy regimes in chronic autoimmune diseases.

Funded by EUFP7 KINACEPT and William Harvey Research Foundation Grants

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P8

CENTRAL AND SYSTEMIC CORTICOTROPHIN-RELEASING FACTOR-INDUCED ANALGESIA MAY BE MEDIATED BY GLUCOCORTICOIDS

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Corticotropin-releasing factor (CRF) is an important regulator of physiological functions and behavior in stress. Analgesia is one of the characteristics of stress reaction and CRF is involved in providing stress-induced analgesia (Filaretov et al., 1996). Exogenous CRF mimics stress effects on pain sensitivity and causes analgesic effect (Lariviere & Melzack, 2000; Yarushkina, 2008). However, the underlying mechanisms remain to be determined. The present study was undertaken to compare the participation of glucocorticoids in central and systemic CRF-induced effects on somatic pain sensitivity in rats under identical experimental conditions.

The participation of glucocorticoids was studied by pharmacological suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis leading to the deficiency of glucocorticoid production as well as an occupation of glucocorticoid receptors by its antagonist RU 38486. Since CRF administration causes the release of β -endorphin from the pituitary, the opioid antagonist naltrexone was used to determine the contribution of opioid-dependent mechanism to both central and systemic CRF-induced analgesia. An electrical current threshold test was applied for measurement of somatic pain sensitivity in anesthetized rats

Both central (2 µg/rat, i.c.v.) and systemic (40 µg/kg, i.p.) administration of CRF caused analgesic effects and an increase in plasma corticosterone level. Pretreatment with naltrexone did not change analgesic effects of central or systemic CRF. Pharmacological suppression of the HPA axis as well as pretreatment with RU 38486 attenuated both central and systemic CRF-induced analgesic effects. However, systemic CRF-induced effects were partially reduced while central CRF-induced effects were completely suppressed by these pretreatments.

The data suggest that the both central and systemic CRF-induced analgesic effects may be mediated by nonopioid mechanism associated with endogenous glucocorticoids in our experimental conditions. At the same time according to data obtained glucocorticoid-independent mechanisms may also contribute to systemic CRF-induced analgesic effects additionally to glucocorticoid-mediated mechanisms.

Acknowledgements: This study was supported by grants from RFBR N 09-04-00964, FNM-2011, DBS RAS (2011).

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Postscript:

FROM MUCOSAL ENERGY METABOLISM TO CAPSAICIN – STRATEGIES FOR UNDERSTANDING MUCOSAL PROTECTION

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Dear Professor Rainsford, Dear Professor Callingham, Dear Participants,
Firstly, I have to apologize because I could not able to come to Cambridge and to participate on the excellent Meeting.

Professor Klara Gyires (Budapest, Hungary) were kindly offered to present the essential points (notes) of my introductory lecture.

1. You have to know that I am clinician (internist), who working in this field from the years of 1960 .Firstly I and (we) was (were) interest to know more and more facts on the drugs actions in patients (including their main actions, side effects, absorption, metabolization and excretion). Were surprised in 1965 , because the duodenal ulcer healed (under clinical conditions) without any decrease of gastric acid secretion. Similar observations were done in multiclinical, randomized, prospective and multicentric study, when the ulcer healing effect in patients treated with atropine, cimetidine and carbenoxolone was superior than that in placebo treated group, however their effects (atropine, cimetidine and carbenoxolone) were the same. These results demonstrated that the decrease of gastric acid didn't necessary for the ulcer healing, therefore the presence of gastric acid secretion does not equal to existence of ulceration.

2. In the 1967-70s years were tried to find the clinical pharmacological explanations of these phenomena, when the development of tolerance (to the drug used in the treatment) and cross tolerance (which never was applied during the treatment, however the chemical structure indicates similarity to the principally used drug) .That was only the one side of the drugs actions, meanwhile the presence of „pharmacologically induced hypersensitivity” was also obtained.

These results were reversible, because these two phenomena („pharmacological denervation supersensitivity” and „tolerance”) appeared together during the chronic treatment, and these phenomena disappeared together after cessation of chronic drug treatment.

3. These results led us to start the biochemical pharmacological studies (earlier in animals, later in patients). We tried to analyze the cross-section on the biochemical building of tissues by the simultaneously carried out measurements of different fractions of phosphorus components, lipids, RNA, DNA (in the years from 1967 to 1972-73). Of course, we applied different animal models and different experimental circumstances (treatment with cholinesterase inhibitors, chemical and surgical vagotomy, different necrotizing agents).

These observations clearly proved that: 1. the chemical and surgical vagotomy produce significantly different metabolic changes in the target organ; 2. we were able to exclude biochemically the presence of tissue hypoxia in the damaged GI mucosa in animal experiments and in ulcerated gastric, duodenal and jejunal mucosa in patients.

4. In 1968-69 I stayed in Norway (Department of Pharmacology, University of Oslo, Norway). Some of the researchers of this institute came home from U.S.A., who worked together with professor Sutherland. His interesting covered the „second messenger system” and received Nobel Price in 1971 for these works. The essential point of Sutherland’s observation was that the plasma membrane located enzyme (adenylate cyclase) split of ATP – in presence of Mg^{2+} into cAMP, and this cyclic adenosine monophosphate will appear in the tissue. This transformation could be modified by different drugs.

From the literature , it’s well know that the regulation of classical „sodium pump” is also an energy dependent process, and the transformation of AP into ADP by Na^+-K^+ -dependent APase is the energy source for the active transport of sodium and potassium across the plasma membrane. This enzyme is also located in the plasma membrane, and the substrate is also the ATP (in presence of Mg^{2+}) and it can be inhibited by ouabain . Skou (Denmark) also received Nobel Price for this discovery(1997). The energy liberates during the ATP splitting into ADP or cAMP, but in not so equal quantities.

Because the ATP is a common substrate for the ATP-ADP transformation by membrane ATPase and ATP-cAMP (in presence of Mg^{2+}) , therefore we deeply analysed the possible correlation between the two membrane bound enzyme systems. These studies were carried out by simple of prepared enzymes, and by the direct and simultaneously carried out measurements of tissue ATP, ADP, cAMP, AMD (and by other components).

It was noted that:

the drugs modified the ATP-cAMP transformation in much more smaller concentrations produced counteract actions on the membrane ATP-ase system

(inhibition vs. stimulation and vice versa);

a very complex system exists between the two enzyme systems regulated by mediators, hormones and drugs;

we were able to demonstrate that the different necrotizing agents produce the same biochemical pathways in the target organ both in development of aggression and protection; the biochemical changes appear earlier in time, than the macroscopic injury, both in the development of injury and protection;

the drugs with absolutely different biochemical mechanisms of action (e.g. atropine, cimetidine) prevent the development of GI mucosal damage..

5. The „cytoprotection”(nominated by André Robert in 1979) represented a new pathway to approach the gastric mucosal protection. We learnt a lot of new things from these studies, which clearly demonstrated the different of chemical molecules produce GI mucosal protection and this phenomenon does not specific to the stomach. The potential mucosal protective role of retinoids was proved in the gastric, gastrointestinal mucosa, protection of drug side effects, inflammatory bowel diseases and cancer development in human beings.

Probably one of the most important discovery was that the intact vagal nerve is basically necessary for the development of gastrointestinal cytoprotection produced by different compounds (1981).

6. The participation of vagal nerve both in the development of aggression and prevention does not questionable.

The vagal nerve contents efferent nerves in about 10 per cent, while the afferent nerve is about 90 per cent from the total.

The modern pharmacological studied proved that the capsaicin-sensitive afferent nerve is also about 10 per cent from the total afferent fibres. Many observations indicated that the capsaicin effect is a dose-dependent process (in small doses stimulates the capsaicin-sensitive nerves , while in high dose impairs these fibres). The capsaicin in small doses prevents the GI mucosa.

Molecular pharmacological studies proved that the preventive doses of capsaicin (which stimulates the capsaicin-sensitive afferent nerves) act in much more smaller doses, than the doses of the classical pharmacologically applied drugs. These results offer us to evaluate more and more suggestions that the capsaicin-sensitive nerves have essential role in the physiological regulation of gastric acid secretion, keeping the mucosal integrity and in the possible mechanisms against produced by different damaging agents. We have to learn more on the cellular mechanisms of capsaicin in GI tract.

Very recently we proved that in patients with Helicobacter pylori positive gastritis, the capsaicin-sensitive responses (TRVP1, CGRP, Substance P) are independent processes from the successfully carried out eradication treatment. *The capsaicin research offers a new pathway to approach the physiological role of gastric acid secretion and to understand the gastric mucosa integrity.*

In summary:

We learnt much more from the earlier carried out observations, however we know very little about the development of aggression and prevention. We couldn't understand the actions of classical drug therapy. We entered into different fashioned research fields (oxygen free radicals, decrease of acid secretion, tissue hypoxia, etc), but our knowledge remained to be unclear.

We emphasised the role of clinical pharmacological studies, GI mucosa biochemistry (cellular energy systems), „gastric cytoprotection”, retinoids, surgical and chemical vagotomy and very recently the capsaicin-sensitive afferent nerves in our research works.

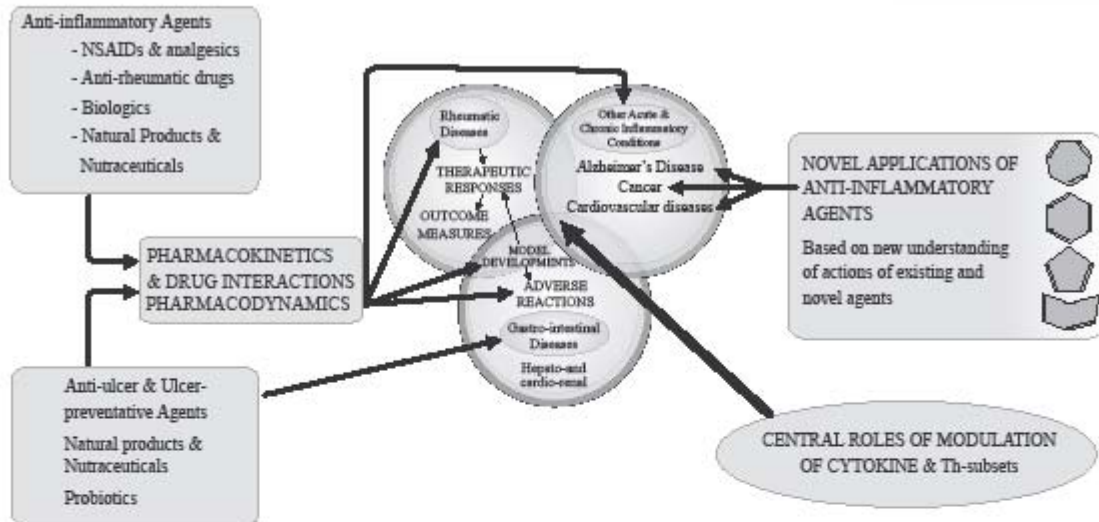
Finally I wish all of you good congress, fruitful discussions and good conclusions for the future. I would like to hope very much that I will have opportunity to meet you personally in the forthcoming time.

June 29th, 2011. Pécs, Hungary.

Gyula Mózsik, MD, PhD, ScD(med)

Thematic Perspective of InflammoPharmacology

'ADVANCING RESEARCH IN NEW MEDICINES'



Inflammopharmacology Conference Series

(With Side-Effects of Anti-inflammatory Drugs Symposia)

Information on Past Conferences 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,
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