Abstracts from Presentations at the SAPS Congress 2004 in Bloemfontein

38th South African Pharmacology Congress,
held at Bloemfontein, South Africa,
24-27 October 2004

South African Pharmacology Society
Suid Afrikaanse Farmakologie Vereniging

Compiled & Updated May 2005

Table of Contents

1 Plenary Lectures

1.1 *Prof W Derman: Drugs in Sport & Therapeutic Use Exemption Certification in 2004*

Drugs in Sport & Therapeutic Use Exemption Certification in 2004

Professor Wayne Derman

The use of illegal performance enhancing substances by elite athletes seems to be uncovered with increasing frequency recently. Indeed, twice as many positive tests were reported during the Olympic Games in Athens 2004 compared to Sydney 2000. Thus, the problems related to drugs in sport have been thrust into the spotlight. The WADA list of banned substances released in 2004 was thought to include medication and rule changes that would make things easier for the team physician. However, the new standards for Therapeutic Use Exemption Certification (TUE) and Abbreviated Therapeutic Use Exemption Certification (A-TUE) have placed a large burden on the team physician.

This lecture will highlight:

1. Ergogenic aid use patterns by athletes.
2. The findings from Athens 2004.
3. Current Therapeutic Use Exemption Standards.
4. Challenges facing the team physician relating to drugs in sport.
5. The supplement and neutraceutical industry: friend or foe?
6. Anticipated 2005 WADA changes to the list of banned agents.

1.2 *Prof IE Hughes:20/20 Vision – Teaching Pharmacology in 2020*

20/20 Vision – Teaching Pharmacology in 2020

I E Hughes

LTSN Centre for Bioscience, School of Biomedical Sciences, University of Leeds, LS2 9JT, UK. i.e.hughes@leeds.ac.uk Tel: +44-113-343-4313

Pharmacology teaching is currently in a state of change because of the large number of pressures which are bearing on academic pharmacologists and their teaching practice. In addition, there is a realization that graduates appropriate to the practice and development of pharmacology in 2020 may need different knowledge, skills and attitudes from those produces in the last decade. Pressure for change comes from: changes in the discipline itself and the discovery of new knowledge and relationships; developments in the tools available for teaching and their application; alternations in expectations and needs regarding teaching styles; changes in the environment in which each university operates and teaches; changes in the nature and expectations of our students and customers; and changes in the needs of academic pharmacologists themselves.
If pharmacology is to deliver an education appropriate to 2020 then pharmacology teachers must be prepared to respond to the pressures for change with appropriate innovations in their teaching practice. Such innovations may be time consuming to develop and implement and academic pharmacologists have other priorities, such as research, to which they must direct their activities. Responses to pressures for change may therefore be best evolved not by individual pharmacology teachers working in isolation, but by collaborative groups at local, national and international levels. Some structure to encourage such collaborative groups of pharmacologists are already in place and need to be fully exploited by pharmacology teachers while the development of other structures may need to be facilitated by national pharmacological societies.

1.3 *Prof IE Hughes: Computer Aided Learning in Pharmacology*

Computer Aided Learning in Pharmacology

I E Hughes

LTSN Centre for Bioscience, School of Biomedical Sciences, University of Leeds, LS2 9JT, UK. i.e.hughes@leeds.ac.uk Tel: +44-113-343-4313

Various types of software have been developed for use in pharmacology courses. These include: simple drill (questions and answer) software; electronic books; video material; tutorial type programs; simulations; and electronic learning environments for course organisation and delivery. These different types of software can be used in different ways to achieve very different learning objectives and gains in teaching efficiency. For example, software can be used: in tutorial and small group teaching; in lectures; to better prepare students for practical work; as a replacement for practicals; to provide options within a limited course structure; to supplement lectures and enable students to work at their own pace; to provide ongoing access to self-assessment throughout a course; to aid distance learning; as remedial teaching and to extend the student learning experience in areas which are too expensive or too time consuming or for which staff expertise does not exist.

Evidence indicates that it is insufficient simply to make computer based learning material available to students. Like a laboratory class, it must be fully integrated into a module of real benefits are to be obtained. Students need to be taught how to learn from compute based learning materials and how to integrate this learning tool in their learning strategy. Teachers need to be supported not only with information about the availability of software but, equally importantly, about how a program can be integrated into a module.

The development and production of exercises and material which integrate programs into a module takes both expertise and a great deal of time. To help teachers with this problem a recent innovation is the availability of Teaching and Learning Resource Packs (TLRPs) from the British Pharmacological Society specifically designed to provide teachers with pre-prepared material with which they can integrate particular CAL packages into their course. Collaboration in the production of such material facilitates the use of CAL materials and greatly improves the quality and efficiency of teaching. Such collaboration is occurring in the UK through the Learning and Teaching Support Network Bioscience Centre (LTSN Bioscience Centre) and through the British Pharmacological Society while similar collaborative networks have been established at a European level (EpharNet, the European Pharmacology Thematic Network funded through the EC Socrates program) and at a World level (through the IUPHAR Teaching Section). The use of such networks to collaboratively develop and to disseminate material designed to integrate software into courses and to establish that its use is effective in teaching and learning should enhance both the effectiveness and the efficiency of pharmacology teaching.

1.4 Prof LH Opie: Cardioprotective Agents and Clinical Application

Cardioprotective Agents and Clinical Application

L H Opie.
Department of Medicine, MRC/UCT Cape Heart Centre, University of Cape Town

The term "cardioprotection" has a broad and general meaning and stretches from drugs that limit coronary artery disease such as the statins, to those that inhibit platelet aggregation, such as aspirin and clopidgrel, to include antianginal agents and blood pressure lowering agents. More specifically though, cardioprotection most commonly describes additional protection against cardiac complications (such as myocardial infarction), beyond blood pressure control. Experimentally the term commonly refers to protection from experimental ischaemic-reperfusion injury as mediated by insulin. Beta-blockers, as a group, give post-infarct protection but there are no data to show that they lessen the incidence of myocardial infarction in those with stable coronary disease. ACE inhibitors, as a group, give post-infarct protection, especially when there is concomitant heart failure. Both beta-blockers and ACE inhibitors help to protect the failing heart against a fatal outcome. Similar or added benefits are obtained with angiotensin receptor blockers (ARBs) and increasing data suggest that aldosterone antagonists are also cardioprotective in the setting of heart failure. Substantial interest has been aroused by the data of two studies, HOPE and EUROPA, in which the ACE inhibitor gave protection against future cardiac events. The role of BP lowering, found in both studies, remains controversial. In EUROPA, the most recent study, the ACE inhibitor, perindopril, protected against coronary events. Hypothetically, it is those ACE inhibitors that are also lipid soluble that act best on the tissue renin-angiotensin system and can be most expected to give better cardioprotection. The hypothesis would explain why ramipril and perindopril have given positive results for cardioprotection whereas lisinopril, only water soluble, has not (results of ALLHAT). This hypothesis would be further supported if the PEACE study, a preventative study with another lipid-soluble ACE inhibitor, trandolapril, is also positive.

In summary, cardioprotection is an important quality that is, nonetheless, elusive to establish in practice. When treating patients for future cardioprotection, it is important to prescribe the agents chosen in the exact doses and manner in which they were successfully given to patients in the trials.

1.5 Prof BH Meyer: Surrogate Endpoints in Clinical Pharmacology

Surrogate Endpoints in Clinical Pharmacology

B.H. Meyer

FARMOVS-PAREXEL, Brandhof, 9324 South Africa

A Surrogate endpoint is a biomarker used as a substitute for a clinical endpoint. Characterization of a biomarker as a surrogate endpoint requires it to be reasonably likely, based on epidemiologic, therapeutic, pathophysiologic or other evidence, to predict clinical benefit.

Accuracy (correlation of the measure with the clinical endpoint) and precision (reproducibility) are further requirements for biomarkers to be useful as surrogate endpoints.
Surrogate endpoints facilitate early go/no go decisions and they facilitate getting the dose right early in the development of a drug. Inappropriate application of surrogate endpoints may lead to wrong decisions regarding the safety and effectiveness of drugs.
Fast track approval for drugs in conditions like stroke, diabetes mellitus, AIDS may be given for marketing of new drugs based on adequate research proving an effect on a surrogate endpoint that is likely to predict clinical benefit. Approval is subject to further study to verify clinical benefit of the drug.
Early PK/PD modelling is possible by using biomarkers and surrogate endpoints are useful in the study of safety of drugs.
Surrogate endpoints can be useful in study of genetics, genomics and toxicity.

1.6 Prof O Pelkonen: The Role of Cytochrome P450 in Chemical and Drug Toxicity

The role of cytochrome P450 in chemical and drug toxicity

O. Pelkonen

Department of Pharmacology and Toxicology, University of Oulu. POB 5000 (Aapistie 5), FI-90014 Oulu Finland (e-mail: olavi.pelkonen@oulu.fi)

The concept of metabolic activation (i.e. formation of reactive metabolites) has been a very useful paradigm in the field of chemical and drug toxicity, because 1) it has provided a mechanistic and enzymatic basis for understanding the initial toxic action of numerous structurally diverse toxicants; 2) it has provided an explanation for the binding of many toxicants to macromolecules; and 3) it has been a crucial bit of knowledge in the attempts to develop in vitro tests for the prediction of toxicity. On the other hand, the primary purpose of metabolism is the conversion of foreign chemicals into such, often more water-soluble, metabolites, which the body can get rid of.
Both detoxication and activation are catalyzed principally by the so called drug- or xenobiotic-metabolizing enzymes, although some activating reactions are catalyzed also by enzymes principally involved in endogenous metabolism. Cytochrome P450 (CYP) forms in families CYP1 through CYP3 are the main enzymes mediating oxidative metabolism of foreign compounds, including bioactivation of many toxicants. The highest concentrations of most CYP forms are found in liver, but they are present in substantial amounts in several extrahepatic organs. Actually many CYP forms are found especially in those tissues that are ports of entry into the body.
Examples of CYP enzymes catalyzing activation/toxication reactions of some chemicals and drugs: CYP1A1, polycyclic aromatic hydrocarbons; CYP1A2, aromatic amines; CYP2A6, nitrosamines; CYP2B6, cyclophosphamide, CYP2D6, NNK; CYP2E1, paracetamol and halogenated solvents; CYP3A4, aflatoxin B1.
The prevailing opinion thus far has been that - for a given compound - metabolic activation is rather hard to predict because it depends on both the structure of the substrate and the specific enzyme involved (in both activation and detoxication). Another hindrance in the prediction of consequences of metabolic activation has been the lack of exact knowledge about specific enzymes involved in the activation reaction and about endogenous and exogenous factors affecting the level and balance of enzymes. In the light of a large number of CYP enzymes, elucidation of activation of chemicals and drugs pose a big challenge. However, recent availability of specific antibodies, diagnostic inhibitors and other experimental approaches, including transgenic mice, have produced a lot of useful information about enzyme-specific activation reactions. These approaches show promise in that it may be possible in the future to predict an individual's ability (even the inherited ability) to activate specific toxicants.

1.7 Prof O Pelkonen: Clinical applications of pharmacogenomics

Clinical applications of pharmacogenomics

O. Pelkonen

Department of Pharmacology and Toxicology, University of Oulu. POB 5000 (Aapistie 5), FI-90014 Oulu Finland (e-mail: olavi.pelkonen@oulu.fi)

There is substantial variability among patients both in therapeutic efficacy and the occurrence of side effects. A substantial proportion of patients will not respond, or respond only partially, when standard doses of the particular drug are administered. As contributing factors, heterogeneity of the disease and such clinical variables as age, gender, diet, co-administration of drugs, renal and hepatic function have been identified. In addition, genetic factors involved in drug disposition (absorption, distribution, metabolism and elimination) or drug action (receptors and signalling pathways) can modify drug response or are risk factors for adverse drug reactions.

With the rapid progress in genomic research, more genes altering drug response will be identified. This knowledge, linked with advances in genotyping technologies, will decrease the cost of genotyping. It has been claimed that with the use of genomic information, we will be able to better predict an individual's likely response to a drug and select the appropriate dose. This would allow achieving the optimal therapeutic response, avoid therapeutic failure and minimise side effects and toxicity. Although a number of genetic polymorphisms responsible for differences in the metabolism, transport and action of drugs have been known for years, this knowledge has not been translated into clinical practice. Only a few polymorphic genes (e.g. CYP2C9, CYP2D6, TPMT) have been more extensively covered in this respect. One of the problems is that most studies have focussed on the consequences of a single gene polymorphism for altered drug response. This approach neglects the fact that drug response phenotype is a complex polygenetic trait with non-genetic factors contributing to the manifestation of the phenotype. Another major limitation which has prevented the use of pharmacogenetic testing in the clinical setting is the lack of prospective clinical trials demonstrating that pharmacogenetic testing can assist in the selection of the appropriate drug and dose for the individual patient. Since drug response usually involves several genes, the positive and negative predictive value of pharmacogenetic testing will be improved by the haplotypes and it is hoped that this knowledge will make it possible to establish pharmacogenomics as an indispensable contributor for optimising drug development and drug therapy.

1.8 Prof P Rasoanaivo: A case study of a chemosensitizing plant in malaria: Strychnos myrtoides

A case study of a chemosensitizing plant in malaria: Strychnos myrtoides

Philippe Rasoanaivo

Malaria Laboratory, Institut Malgache de Recherches Appliquées, BP 3833, 101-Antananarivo, Madagascar E-mail : rafita@wanadoo.mg

The shortage of standard antimalarials during the sudden recrudescence of malaria in the Central Highlands of Madagascar in 1982's led the population back to the massive use of medicinal plants. Among these, *Strychnos myrtoides* was used to enhance chloroquine action in chronic malaria. After confirmation of the chemosensitizing activities in experimental models (1), then clinical observational study (2) and basic pre-clinical investigations (3-8), a standardized extract was submitted to a controlled, double blind randomized clinical trial (chloroquine + phytomedicine *versus* chloroquine + placebo) in a primary health care of a malarious region. First, the study of the efficacy of chloroquine showed that 24.5% of therapeutic failure and 2 cases of R-III resistance were observed in malarious patients (9). Then 357 patients were enrolled in the clinical trial, in which there was no significant difference between placebo and treated patients (10). However, from 254 blood samples collected from patients, only 4 isolates were resistant with an IC_{50} > 100 µg/ml, and this is in line with the finding that the extract was found to be active against resistant strains (5). A follow-up of the *in vitro* susceptibility of isolates from patients with therapeutic failure revealed unexpected results. At this point, although the IC_{50}s were in the range of those corresponding to sensitive isolates, concave curves were observed in isobologram of drug interaction, clearly suggesting that isolates were mixture of sensitive and resistant strains. Madagascar is therefore in the way of acquiring generalized chloroquine resistance by progressive selection of resistant strains if chloroquine treatment is pursued. In the fundamental aspect, the on-going investigation has shown that the bioactive constituent named malagashanine appears to be a useful biochemical tool that may contribute to the understanding of the mechanism of chloroquine resistance and its reversal. Furthermore, a chemosensitizing pharmacophore was also identified and synthesis of several derivatives is underway.

References

1. P. Rasoanaivo, S. Ratsimamanga-Urverg, R. Milijaona, H. Rafatro, C. Galeffi, M. Nicoletti. *Planta Med*. 1994, 60, 13.
2. A. Ramialiharisoa, J. Ranaivoravo, S. Ratsimamanga-Urverg, P. Rasoanaivo, A. Rakoto-Ratsimamanga. *Rév. Méd. Pharm. Afr*. 1994, 8, 123.
3. M.-T. Martin P. Rasoanaivo, G. Palazzino, C. Galeffi, M. Nicoletti, F. Trigalo and F. Frappier. *Phytochemistry*1999, 51, 479.
4. D. Ramanitrahasimbola, S. Ratsimamanga-Urverg,, P. Rasoanaivo and A. Rakoto-Ratsimamanga. *Phytomedicine* 1999, 6, 331.
5. H. Rafatro, D. Ramanitrahasimbola, P. Rasoanaivo, S. Ratsimamanga-Urverg, A. Rakoto-Ratsimamanga and F. *Biochem.Pharmacol.* 2000, 59, 1053.
6. H. Rafatro, P. Rasoanaivo, S. Ratsimamanga-Urverg, J. Quetin-Leclercq and R. K. *J. Chromato. B* 2000, 744, 121.
7. H. Rafatro, R. K. Verbeeck, P. J. M. De Longhe, Rasoanaivo, A. Laurent and G. *J. Mass Spectr.* 2000., 35, 1112.
8. P. Rasoanaivo, G. Palazzino, C. Galeffi and M. Nicoletti. *Phytochemistry* 2001, 56, 863.
9. M.L. Willcox, E. Rakotondrazafy, R. Andriamanalimanana, D. Andrianasolo, P. Rasoanaivo. *Tr. R. Soc. Trop. Med. Hyg.* 2004, 98, 311.
10. D. Ramanitrahasimbola, J. Ranaivoravo, H. Rafatro, P. Rasoanaivo, S Ratsimamanga-Urverg. In: Willcox ML, Bodeker G, Rasoanaivo P (Eds), Traditional Medicinal Plants for Malaria. London: Taylor & Francis 2004 (in press).

1.9 Prof D Steyn: Important side effects of commonly used anti-retroviral therapy (ARVT)

Important side effects of commonly used anti-retroviral therapy (ARVT)

D. Steyn

Department of Internal Medicine, University of the Free State

Side effects are very common and they should be anticipated, recognized and managed. Patients need to be well informed and management strategies need to be planned on an individualize basis.

Most important side effects of commonly used NRTI's

AZT - Zidovudine	3TC -lamivudine	d4T –Stavudine	ddI - Didanosine	Abacavir - ABC
Macrocytosis Headache Fatigue GIT symptoms Insomnia Hyperpigmentation of nails Myopathy (usually develops after 6-12 months of therapy) Lactic acidosis Bone marrow suppression - Anemia (usually develops after 2-4 months) - Neutropenia	Minimal toxicity Headache Diarrhea Nausea Abdominal pain Insomnia Lactic acidosis and steatosis is less common than with other NRTIs	Asthenia Headache Malaise Insomnia Gastrointestinal intolerance Lipoatrophy Hepatic steatosis Peripheral neuropathy Ascending motor weakness Lactic acidosis	Must be taken on an empty stomach GI intolerance (bloating, flatulence, nausea, diarrhea) Elevated liver enzymes Peripheral neuropathy Pancreatitis Lactic acidosis	Hypersensitivity reaction during the 1st 6 weeks occurs in 3% of cases. It can be fatal so avoid rechallenge. GIT symptoms Lactic acidosis and steatosis is less common than with other NRTIs

Most important side effects of commonly used NNRTI's

Nevirapine - NVP	Efavirenz – EFV
Nausea, diarrhea, headache Rash: usually during 1st 6 weeks non severe in up to 15% - 25% of patients severe in 7% of cases Stephen Johnson syndrome - rare Hepatitis first 6 months needs close monitoring especially for patients with pre-existing liver disease Moderate inducer of P450	Mood disorders (50%) Rash: usually during the 1st 2 weeks on treatment non severe in up to 17% of patients severe in 1,7% of cases Hepatotoxicity is less frequent and less severe than with NVP Fetal malformations Mixed P450 inhibitor & inducer

Class adverse reactions of all PIs

High pill burden
Drug interactions
Fat redistribution or lipodystrophy
Metabolic disorders (insulin resistance with hyperglycemia, lipid abnormalities, osteopenia)
Food restrictions with some

Lopinavir / Ritonavir – generally well tolerated (combination PI in the South African ARV program)
Indinavir – renal stones, asymptomatic increase in indirect bilirubin
Nelfinavir – diarrhea
Ritonavir – bad taste, GIT intolerance & hepatotoxicity is more severe and more common than with other PIs
Saquinavir - soft gel or hard gel formulation
Amprenavir – once daily dosing if combined with ritonavir
Atazanavir - once daily and less hypercholesterolaemia
Tipranavir - possible role in salvage therapy

Summary

The side effects of ARVT, the high pill burden and the need for an almost 100% adherence greatly impair on the quality of patients' lives. Combination tablets as well as tablets that can be taken once daily is becoming more popular and it may eventually become the standard in HIV care.

Reference: Bartlett J.G, Gallant J.E; Medical Management of HIV infection 2003.

1.10 Prof A Walubo: Clinical Pharmacology In Developing Countries; Defining A Discipline

Clinical Pharmacology In Developing Countries; Defining A Discipline

A. Walubo

Department of Pharmacology, University of the Free State, South Africa.

The profession of 'Clinical Pharmacology' developed as far back as in the 1930s as a result of increased concern for drug safety. But it was only in 1970 that the WHO technical report defined the functions of clinical pharmacology as, "To improve patient care by promoting safer and more effective use of drugs; to pass on knowledge through teaching and to provide services, such as drug information, drug analysis, the monitoring of drug abuse and advice on experimental design of clinical studies. All these functions should in fact serve to enhance benefit-cost ratios of drugs". Although this definition is still valid, different fields of clinical pharmacology have been emphasized at different times in relation to the society's needs. In the 1960s, emphasis was on controlled clinical trials, drug metabolism and clinical pharmacokinetics, while in the 1970s there was shift towards pharmacogenetics, therapeutic drug monitoring and drug utilization research. In the 1980s, the emphasis was more on pharmaco-epidemiology, pharmacovigilance and drug information, while the last decade (1990s) the attention has been to molecular pharmacogenetics and pharmacogenomics. This has been associated with development of educational programs for training of specialized clinical pharmacologists in each of these fields.

Unfortunately, this was not the same for developing countries where the majority of the population rely on traditional medicines due to lack of access and affordability for the synthetic drugs. To these people, the trend has been traditional medicine, traditional medicine and traditional medicine. This trend was somehow influenced by the WHO which, in 1970s emphasized the 'essential drug' concept, but in the current decade, their emphasis has shifted back to 'traditional medicine'. This is because, even when synthetic drugs are available, their rational use is difficult owing to lack of support structures such as drug information services, therapeutic drug monitoring, testing safety standards, effective drug policies, appropriate clinical pharmacologists, etc. The 'essential drug' concept enabled countries to focus their limited resources on cost-effective drugs that serve the needs of more than 90% of their population. Since then, clinical pharmacology in developing countries has been concerned with improving access, affordability and appropriate use of medicines. It promotes the use 'essential drugs' and 'traditional medicines' to minimize cost, increase affordability and access, and improve drug safety through guidelines on rational prescribing. It requires a collective effort of different professional groups, including managers and traditional healers; inclusion of clinical pharmacologists in therapeutics committees; use of appropriate qualitative and quantitative research methods in drug use reviews; use of public health empowerment techniques; evaluation of clinical trials and engagement with basic pharmacologists. Unfortunately, there is no medical school that offers such training, and this calls for development of a purposeful clinical pharmacology curricular for developing countries which should include a strong component of traditional medicine and public health principles, a new discipline.

1.11 Prof JES Wikberg: Proteochemometrics: A Bioinformatics Approach to Drug Discovery

Proteochemometrics: A bioinformatics approach to drug discovery

J.E.S. Wikberg

Department of Pharmaceutical Biosciences, Uppsala University, Box 591, SE-751 24 Uppsala, Sweden. Email: Jarl.Wikberg@farmbio.uu.se

The function of living matter is determined by its structure and organisation, and the interactions by its constituents. The compositions of living matter is ultimately determined by the sequences of the genomes. The advent of complete genome sequences of several species has brought great hope to bring an ultimate understanding of the processes of life. However, understanding the function of a gene and its role in a cell will need a greater step than was taken in arriving to its sequence.

The number of genes in the human genome is estimated to about 30-40,000, while the number of proteins in the human proteome may be 200,000 – 2 million, due alternative splicing, posttranslational processings and varying subunit assembly. The genomes also contain substantial genetic variations. In drug-discovery we desire to map the interactions of organic compounds (drugs and metabolites) with the proteome. Since organic compounds with molecular weight less that 500 Da is estimated to 10^{200}, of which 10^{60} may be 'drug-like', the task of such mapping is astronomic and will surely never happen without a proper technology that can *a priori* select the relevant combinations of interacting entities for experimental evaluations, and without computational methods that can correctly predict the functions of the biomolecules, and their interactions with the surrounding.

While previous methods to molecular recognition required access to protein 3D-structure, which requires extensive resources to arrive at, we recently developed a new bioinformatics approach suited for drug design, protein engineering, functional genomics analysis, and mapping molecular recognition [1]. This technology, which we have termed *proteochemometrics*, does not require knowledge of the 3D-structure of the bio-macromolecules and applies statistical bioinformatics modelling on interaction data derived from simple readily available standard biochemical assay methods. The data gathered so far demonstrate the utility of proteochemometrics for detailed mapping of molecular recognition (i.e., down to the physicochemical amino-acid level of drug-targets), for remarkably accurate *a priori* predictions of the binding strength of novel candidate-drugs, and for *a priori* predicting the behaviour of proteins subject to artificial mutations.

The lecture will explain the principles of proteochemometrics and demonstrate its use in a number of selected cases.

1. Wikberg, J.E.S., M. Lapinsh, P. Prusis: Proteochemometrics: A tool for modelling the molecular interacitons pace. In: H. Kubinyi, G. Müller, eds. Chemogenomics in Drug Discovery - A Medicinal Chemistry Perspective, Weinheim, Wiley-VCH, 2004, p. 289-309.

1.12 Prof JES Wikberg: Melanocortin receptors: New opportunities in drug discovery

Melanocortin receptors: New opportunities in drug discovery

J.E.S. Wikberg

Department of Pharmaceutical Biosciences, Uppsala University, Box 591, SE-751 24 Uppsala, Sweden. Email: Jarl.Wikberg@farmbio.uu.se

Slightly more than a decade ago a new class of G-protein coupled receptors, the melanocortin receptors were cloned. In mammals the class contained five subtypes MC_{1-5}. Of these the $MC_{1,3-5}$ receptors turned out to be responsive for the naturally occurring melanocyte stimulating hormone peptides α-, β- and γ-MSH, while the $MC_{\square}$ receptor was found to be the ACTH receptor controlling the corticosteroid production of the adrenals. In the following years the physiological function of the MSH-responsive melanocortin receptors started to become understood. While it was early clear that the MC_1 receptor controlled pigment formation of the melanocytes it was later shown also to have a profound role in modulation the immune system, with the receptor being present on distinct subpopulations of peripheral lymphocytes, and its activation leading to strong anti-inflammatory responses in various models of inflammation. The MC_3 and MC_4 receptors were discovered to be present in distinct regions of the central nervous system and both of these receptors were strongly implicated in the control of food intake behaviour and of sexual behaviour. Moreover, the MC_3 receptor was later discovered on immunocompetent cells and as well implied in the control of the immune system. The MC_5 receptor at last is present in many peripheral locations and has a role in control of exocrine gland function.

Due to their physiological functions the MC receptors became desired targets in drug development. In particular it has been hoped that drugs can be developed for treatment of sexual dysfunctions and of obesity based on MC_4 and/or MC_3 agonistic profiles, and that novel antiflammatory therapies can be based on compounds with an MC_1 agonistic profile. However, despite quite intensive research only few low-molecular weight compounds have emerged that have distinct potential as orally active remedies with MC-receptor activity. Moreover, there exists unclarity as to which MC receptor subtype that should actually be activated to elicit a desired effect; in case of sexuality the MC_4 or the MC_3, and in case of inflammation therapy the MC_1 or the MC_3?

In our recent research to understand the molecular pharmacology of the MC-receptors we have found strong evidence that the MC receptor function is not as simple as earlier anticipated, being presumed to being monomeric G-protein coupled receptors coupling to G_s, thereby causing stimulation of adenylyl cyclase and formation of cAMP. Rather we have obtained concerted evidence that the MC receptors are capable of forming both homo- and heterodimers, and that such receptor aggregates interact causing both negative and cooperative effects on the binding of drugs to the receptor sites. Such interactions opens the possibility of cross-talks between the different MC-receptorsubtypes, and may be a reason for the confusions in the understanding of their physiology, as well as a factor of serious consideration in any drug development project for these receptors.

The lecture will summarize aspects of our current knowledge of the MC receptors and attempts to design drugs for them.

2 Oral Presentations

Treatment costs for HIV/AIDS in a managed care setting in South Africa

FJ Arens[1], MS Hislop[2], E Sinanovic[3], L Regensberg[2], G Maartens[1]

[1]Division of Clinical Pharmacology, University of Cape Town, [2]Aid for AIDS (Pty) Ltd., [3]Health Economics Unit, School of Public Health, University of Cape Town

Background: Studies in industrialised countries show that expenditure on HAART is offset by savings on hospital costs. Here we report treatment costs for patients on HAART who are beneficiaries of medical schemes contracted to Aid for AIDS, an HIV/AIDS managed care company

Methodology: This study retrospectively analyses claims authorized for payment by adults who initiated HAART between 1998 and 2003. Treatment costs were categorised into consultations, hospitalizations, investigations and medication.

Results: Treatment costs are highest in the 3 months prior to registration reflecting a large proportion of patients who have HAART authorised following hospitalisation. Thereafter expenditure decreases before reaching a plateau, which remains stable for 36 months. Subgroup analysis by CD4 strata shows that peak treatment costs at entry are approximately double for patients who commence HAART with entry CD4 counts< 200 cells/µl compared with ≥ 200 cells/µl.

Conclusions: Price reductions have made HAART affordable. Average treatment costs for patients on HAART are stable in the 36 months post entry in the context of a managed care programme. Commencing HAART with CD4 counts < 200 cells/µl is associated with high initial expenditure.

CYP2D6 polymorphisms in Southern African populations: Equal treatment for all?

C. Coetsee and G.H.J. Pretorius.

Dept of Haematology and Cell Biology, Faculty of Health Sciences, University of the Free State, P.O. Box 339. Bloemfontein 9300, South Africa

Debrisoquine 4-hydroxylase, also known as CYP2D6, is a cytochrome P450 enzyme responsible for the metabolism of many commonly used drugs such as neuroleptics, beta-blockers, opiates and tricyclic antidepressants. Polymorphic expression of CYP2D6 is observed in the interethnic and inter-individual variability in patients undergoing treatment with these drugs. Two distinct phenotypes have been described for this enzyme in Caucasian populations, namely extensive (EM) and poor metabolisers (PM). Major discordance has been observed when comparing Caucasian data with data from other ethnic groups. Accurate prediction of a patient's genotype could be important for many clinically used drugs as poor metabolisers are at a high risk for adverse drug reactions, onset of toxicity and even therapeutic failure. Early or preventative therapy guided by genotyping could significantly enhance the clinical outcome for these patients.

In this study we determined the frequency of CYP2D6 polymorphisms in three Southern African populations, namely Caucasian (control), Black and Coloureds. Our results for the Caucasian group largely confirm the findings of other studies in the sense that 96% of subjects are EM (normal). However, based on their genotypes, only 70% of Coloured and 69% of Black subjects are predicted to be EMs, the balance being intermediate (IM) or PM. Both groups also show reduced-activity alleles unique to them, not found in the Caucasian subjects. If confirmed by phenotyping of subjects on a relevant treatment regimen, these findings may have implications for management of patients with drugs metabolised by CYP2D6.

In vitro absorption of humic acids from rat gastrointestinal tract

A.D. Cromarty and C.E.J. van Rensburg

Department of Pharmacology, University of Pretoria, PO Box 2034, Pretoria, 0001. duncan@med.up.ac.za

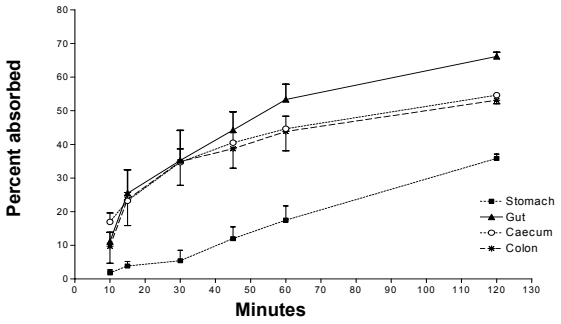
Humic acids are derived from plant material and are ubiquitous in nature. Although the chemical structure is unknown they are reputed to have molecular masses greater than 10Kda, a factor that could limit absorption from the gut.

Humic acids have been reputed to have anti-inflammatory effects and have been demonstrated to have inhibitory effects on CR3 adhesion molecule expression *in vitro*.

The aim of this study was to confirm whether humic acids could be absorbed from the GIT in therapeutically valid concentrations.

Diet restricted adult SD rats were euthanased and the entire GIT dissected out and segmented into the stomach, small intestine, caecum and colon. Each segment was washed internally with glucose containing PBS and filled with 2ml HBSS and 0.5ml ^{125}I-labelled humic acid and closed off at each end with suture silk. The segments were quickly washed in PBS before incubating in HBSS at 37°C. Two ml samples were removed at 10, 15, 30, 60 and 120 minutes. These aliquots were analysed for radioactivity. The final suspending medium from each GIT segment was dried and resolubilised in methanol, acetone or ammonium hydroxide solution and analyzed by HPLC.

Typical time and dose curves were obtained for the absorption of humic acids from the GIT and summarised in Figure 1. HPLC analysis showed dark coloured, late eluting peaks in addition to several early eluting peaks. It can be concluded that humic acids can be absorbed from the GIT. It is possible that part of the humic acids are bound to proteins or mucins.



The Effect of the Extracts of Schkuhria pinnata on the Growth of Escherichia coli Strains on Nutrient Agar Plates.

PJ Yengkopiong, CP Kahler and WJ du Plooy.

Department of Pharmacology and Therapeutics, P O Box 225, MEDUNSA, 0204, South Africa. Email: jadalojuka@hotmail.com

Extracts of *Schkuhria pinnata* are used by the *Pedi* to treat stomach cramps. Stomach cramps are due to ingestion of contaminated food or bacterial infections that result in increased parasympathetic stimulation of the GI tract causing colicky pain or diarrhea or both. Infectious *E. coli* strains are emerging to cause diarrhea, urinary infections, sepsis or meningitis. Seven serogroups that vary in their virulent genes have been categorized. These are the Diffusely adherent-, Enterotoxigenic-, Enteropathogenic-, Enteroinvasive-, Enteroaggregative- and Shigatoxin producing *E. coli*. These are resistant to antimicrobial agents. This calls for a search of new antimicrobial agents and it is in this that we partake. Ten *E. coli* serogroups, nine of which contained either of the genes and one unknown, were used in the study. The strains were streaked on nutrient agar plates. 5µl of the plant extracts from the leaves, stem or roots with distilled water or 70% ethyl alcohol made at 0, 3, and 24 hours was inoculated on specific spots on the agar. These were incubated at 37 ^{0}C for 72 hours. The growth pattern was monitored at 24, 48 and 72 hours. There was a general growth inhibition of all the *E. coli* stains within 24 hours. After 48 hours, growth resumed in those areas of water extracts only. In the control, ethyl alcohol inhibited the growth of some strains within 24 hours; however the growth resumed thereafter. This confirms a potential benefit of the plant extracts in bacterial control. It also provides a rationale for the use of the plant preparations by the *Pedi* to treat stomach cramps and diarrhea. To our knowledge this is the first scientific investigation of the extracts of this plant to be reported. This opens a new opportunity to identify the plant component with the antibacterial property.

Is searching for high activity antimicrobial compounds from plants an exercise in futility?

JN Eloff

Programme for Phytomedicine, Dept of Paraclinical Sciences, University of Pretoria, South Africa

A survey in 1984 found that at least 25% of prescriptions in the USA and Canada contain bioactive compounds derived from plants or modeled after plant natural products. There have been hundreds of publications on screening plant extracts for potent new antimicrobial compounds. Yet there has been very little success in developing a product to enter the pharmaceutical market.

Based on our work in isolating anti-infective compounds from the Combretaceae we have identified the following possible explanations.

The agar diffusion bioassay method used by most scientists usually does not work well with plant extracts and there is little correlation between minimum inhibitory concentrations (MIC) values and inhibition zone diameter.

The extractants used frequently do not extract the antimicrobial compounds.

Many scientists have followed ethnobotanical leads, in our experience most antimicrobial compounds are non-polar, traditional societies do not have non-polar extractants available. Scientists have therefore followed the wrong leads.

The evidence is growing that in many plant extracts there synergistic effects occur Isolating a single active compound frequently does not increase activity as much as can be expected.

It is difficult to compare results of different scientists because different strains are used for evaluation and there is no agreement on what constitutes an active extract. Many small laboratories start screening plants for especially antibacterial activity and consider MIC values of 5 mg/mL as proof of antibacterial activity.

In many cases promising data is not followed up because it requires specialized expertise.

All of these aspects will be illustrated from our results in screening and isolating antibacterial and antifungal compounds from *Combretum* and *Terminalia* species in South Africa. By applying methods that we have developed for extraction and bioassay and by screening plants on a random base we have found that crude leaf extracts of several trees had MIC values as low as 20-40 µg/mL towards e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis, Candida albicans, Cryptococcus neoformans* and *Mycobacterium smegmatis.*

By applying the methods we have used and screening plants widely the chance of identifying new magic bullets from plants may be increased.

Analysis of cognitive knowledge in MEDUNSA BPharm examinations by the use of Bloom's Taxonomy

S. Fourie*#, B. Summers#, J.C. Meyer#, C.O. Haavik$^{\Delta}$, P. Richards$^{\blacklozenge}$.

* Department of Pharmacology, Box 225, MEDUNSA, 0204, South Africa

(e-mail: sfourie@medunsa.ac.za), # School of Pharmacy, MEDUNSA, 0204, South Africa

$^{\Delta}$ University of Botswana, Private Bag UB 00706, Gaborone, Botswana

$^{\blacklozenge}$ Khulisa Management Services, 26 7th Avenue, Parktown North, Johannesburg, South Africa.

Educational health programmes are challenged to ensure the quality and credibility of their assessments. The aim of this study was to investigate the quality of written knowledge assessment methods employed in the fully integrated, PBL BPharm programme presented by MEDUNSA in cooperation with Tshwane University of Technology (TUT). Bloom's Taxonomy was used to grade the depth of cognitive knowledge in the assessments.

Method: The BPharm written examination papers consist of a T/F section and a section that contains short written, construct-response questions. Two senior lecturers of the School of Pharmacy analysed by consensus the depth of knowledge of questions in the latter sections of the papers presented at MEDUNSA. A grid was used to link the marks allocated to each question proportionally with one or more of the six levels of Bloom's Taxonomy. The total percentage of marks in each Bloom's level was calculated and percentages were combined in groups as follows: 1&2 (knowledge and comprehension); 3&4 (application and analysis); and 5&6 (synthesis and evaluation). A ratio was calculated for the percentages in each combination.

Results: The depth of knowledge in the questions of 25 End of Module (EOM) and 15 End of Semester (EOS) examinations written from 1999 to 2003, was analysed. The overall distribution of questions across the six levels of Bloom's Taxonomy showed a mean ratio of 5:4:1 in levels 1&2:3&4:5&6. The mean ratios for questions in the EOM examinations for the different academic year groups were: first year 5:4:1; second year 5:5:0; third year 5:5:0 and fourth year 5:4:1. The mean ratios for questions in the EOS examinations for the academic year groups were: first year 5:4:1; second year 5:4:1; third year 5:5:0 and fourth year 4:4:2. Determination of these ratios is a new approach to analysis of the depth of knowledge of questions in BPharm examinations.

In conclusion, the mean ratios across the levels of Bloom's Taxonomy found for the first and second year EOM examinations are considered to be acceptable for the MEDUNSA/TUT BPharm programme. More questions in the higher levels (5&6) of Bloom's Taxonomy in the third and fourth year EOM examinations could improve the programme. It is considered that the third year EOS examinations should have more questions in the higher levels of Bloom's Taxonomy. The progress shown in the depth of EOS questions from the first two years to the final year is considered as a positive finding.

Nutritional supplements - the South African scenario

E Grobbelaar, PJ van der Merwe.

South African Doping Control Laboratory, Department of Pharmacology, University of the Free State, Bloemfontein, South Africa.

Inadvertant doping through the use of nutritional supplements is becoming commonplace in South Africa. Several studies in Europe and the United States have shown that nutritional supplements contain prohormones not listed on labels and that the use of such products may lead to a positive doping control test. In the first part of the study, locally bought over-the-counter nutritional supplements was screened for the presence of prohibited substances according to WADA's prohibited list. The influence of the intake of contaminated nutritional supplements (identified in the first part) on the urinary steroid profile of healthy male volunteers was also investigated.

The supplement samples were screened qualitatively by gas chromatography/mass spectrometry for testosterone and its prohormones and nandrolone and its prohormones according to Geyer *et al*. A gaschromatograph with a nitrogen-phosphor detector was used to screen samples were deemed necessary for caffeine and ephedrine's.

Sixty two (62) different nutritional supplements from 25 different manufactures bought in local shops, were tested. Forty three (69%) of the 62 supplements contained no prohibited substances. Of the 19 (31%) positive supplements, 10 (52.6%) contained prohormones and nine (47.4%) contained stimulants. Seven supplements contained prohormones, which were listed on the labels, while 3 supplements contained prohormones, not listed on the product labels. Stimulants were only listed as herbal extracts.

One capsule of a supplement containing 19-nor-4-androstenedione and 4-androsten-3,17-dione not listed on the label was administered to healthy male volunteers. The results of the excretion study showed that after the intake of amounts lower than the recommended dose, athletes can fail a doping test for longer than 24 hours post administration.

Incremental crataegus supplementation: symptomatic and objective outcome in patients with early cardiac functional impairment.

P.J. Jordaan, B.H. Meyer, S.G Pretorius, L. Potgieter, J. Terblanché, J. H. Potgieter, D Zeh*. Farmovs-Parexel, Private Bag X 09, Brandhof, Bloemfontein 9324 & *Duopharm GmbH. (Pierre.Jordaan@farmovs-parexel.com)

Recent treatment modalities for cardiac failure improve both morbidity and mortality. Naturopaths have prescribed Crataegus for circulatory and cardiac conditions for almost 2000 years. Redefining the role of Crataegus' adjunctive role, if any, in this context was the aim of two consecutive studies in patients with stable NYHA I and II impairment.

Patients (n=45) received standard Protecor® (contains 50-mg Crataegus) or placebo TID for 12 weeks in a randomized, placebo controlled crossover study. Consenting patients (n=35) then received Protecor® Forte (200-mg Crataegus) TID in an open study for a further 48 weeks. All patients took standard background medication. Function evaluation included Bruce protocol exercise stress test (ETT) and non-invasive cardiac output at baseline, after 12 weeks Protecor® or placebo and after 12 and 48 weeks Protecor® Forte treatment.

No significant safety or efficacy effects occurred at 12-week Protecor® therapy. After 12 weeks vs 48 weeks of Forte treatment exercise duration increased by 1 minute in 23% vs 31%, and RPP increased by 10% in 31% vs 38% (compared to 22% for ETT and 27% for RPP after 12 weeks Protecor®). Other findings at 12 vs 48 weeks on Forte were: absence of angina in 66.7% vs 76.9%; normalized functional capacity in 2.6% vs 35.9%; marked improvement in quality-of-life rating in 10.3% vs 46.2%. Mean ECG changes for resting pulse, QRS and QTc were –1 bpm, + 6ms and + 8ms respectively. No significant treatment-related side effect trends were documented.

Summary: This data reflects incremental improvement of both objective and perceived parameters of cardiac function and well being with increased dosage and exposure time with an acceptable side-effect profile against the background of a progressive life-threatening condition where deterioration would have been the norm.

Prevalence of disease states and treatments in private primary health care settings in South Africa

N.L. Katende-Kyenda*[1], M.S. Lubbe[2], J.H.P. Serfontein[3], and I. Truter[4].

[1]Dept of Pharmacology, University of Transkei; [2-3]Pharmacy Practice, North West University; [4]Dept of Pharmacy, University of Port Elizabeth, South Africa (e-mail: kyendanorah@yahoo.com).

The aim of this non-experimental, quantitative, retrospective study was to analyze the incidence of disease states in private primary health care settings and their treatments.

Data were obtained from the central database of a private primary health care provider. The study population consisted of all patients who visited nine clinics situated in different geographical areas of South Africa, from 1st January to 31st December 2001. Disease states were analyzed according to: number of patients, gender, age groups and treatments.

A total of 140723 disease states were diagnosed in 83655 patients. A total of 515976 medicine items costing R1716318.90 were prescribed, of which 18.69%, (N = 96423) were antimicrobials costing 60.89%, (R1045108.00). The most frequently diagnosed disease states were: upper respiratory infection, viral influenza, acute bronchitis, common cold, cough and acute-severe sinusitis. One form of respiratory tract infection was one of the top five disease states. Antibiotic treatments prescribed were penicillins (amoxycillin), sulphonamides (co-trimoxazole) and tetracyclines (doxycycline).

Antibiotics prescribed for respiratory tract infections were neither necessary nor appropriate because most of these infections are caused by viruses and thus are self-limiting. This could indicate overuse and inappropriate use of antibiotics and these are factors often implicated in the development of resistance among causative pathogens of respiratory tract infections.

Occupational post-exposure HIV prophylaxis

Gary Maartens

Division of Clinical Pharmacology, Department of Medicine, University of Cape Town gary@curie.uct.ac.za

The risk of a healthcare worker acquiring HIV following percutaneous occupational exposure is 0.3%. The risk following mucous membrane exposure is 0.09%. An uncontrolled study found that zidovudine post-exposure prophylaxis (PEP) reduces the risk of acquiring HIV by 80%. In many instances PEP is not indicated – e.g. the material the healthcare worker was exposed to is not infectious, exposure to intact skin, source patient HIV negative or healthcare worker HIV positive. The current approach to post-exposure prophylaxis (PEP) is to stratify the exposures by risk and to treat accordingly, with two nucleoside analogue reverse transcriptase inhibitors augmented with an additional antiretroviral agent for high risk exposures. These regimens are empiric and extrapolated from data on treating HIV infected individuals. PEP is not well tolerated with adverse events occurring in about half and therapy discontinued in about a third – the highest rates of adverse events occur with 3 drug regimens. Life-threatening adverse events are rare. PEP should be commenced as soon as possible after the injury. Animal data suggests that prophylaxis after 24 hours is ineffective, but current guidelines allow PEP for up to 7 days after exposure in high risk exposures. Laboratory monitoring is done to exclude acquisition of HIV infection and, for those given PEP, to monitor toxicity. Healthcare workers should be tested for HIV infection at the time of the exposure & again at 6 weeks, 3 months & 6 months. The test of choice is the HIV antibody test, which should be done in a laboratory rather than with a rapid test. Occupational HIV exposure is extremely stressful and there is a need for psychosocial support. Healthcare workers should be instructed to practice safer sex until their HIV test is negative 6 months following exposure. Nucleic acid amplification tests should not be done serially to assess whether HIV was acquired as these tests have a significant false positive rate.

Therapeutic efficacy of sulfadoxine-pyrimethamine alone and in combination with artesunate in uncomplicated malaria in southern Mozambique.

M Machai*#, F Little*, E Allen*, T Camba#, E Streat#, K Barnes*

*Division of Clinical Pharmacology, University of Cape Town, 7925, South Africa.

Provincial Ministry of Health, Av. Gabriel Texeira 438, Matola, Mozambique.

Artemisinin-based combinations are increasingly being adopted to improve cure rates, delay resistance and reduce malaria transmission. We compared the therapeutic efficacy of sulfadoxine-pyrimethamine (SP) monotherapy with SP administered with a 3-day artesunate regimen (AS-SP).

During 2002, patients with uncomplicated malaria were enrolled into a single-arm SP efficacy study in Namaacha (n=100) and Bela Vista (n=49). During 2003, patients enrolled in Namaacha and Catuane were randomised to receive either SP (n=62) or AS-SP (n=66). Patients' response to treatment was followed up for 42 days.

Therapeutic efficacy of SP varied across study sites, ranging from an adequate clinical and parasitological response rate (ACPR) of 71% in Bela Vista to 100% in Catuane. ITT analysis of patients enrolled in all studies found a lower failure rate among those treated with AS-SP compared with those treated with SP monotherapy; RR 4.69 (1.51-14.61; p=0.003). Survival analysis comparing only those enrolled in the 2003 RCT showed superior ACPR rates for patients on AS-SP but this difference was not significant. However, after adjusting for age and parasite density, treatment group was a significant determinant of treatment outcome (p=0.008).

Artesunate plus SP resulted in a higher adequate clinical and parasitological response rate than SP monotherapy in southern Mozambique.

Traditional medicines – an alternative perspective

O Meissner

Department of Pharmacology, University of Transkei, Private Bag X1, Umtata, 5117 South Africa e-mail: meissner@getafix.utr.ac.za

When hearing the word 'traditional' medicine the image of 'herbal' remedies usually springs to mind. Herbal medicines are widely used in developing countries and are gaining worldwide in popularity. A significant percentage of modern pharmaceuticals are derived from plant sources, and new affordable therapeutics are expected to emerge through extraction, isolation and identification of active ingredients of plant materials. The present study was undertaken to gain a better understanding of the traditional medical armamentarium as used in African traditional practice.

Information was gathered through a comprehensive literature review as well as personal interviews with practicing traditional healers in the former Transkei. More than 30 herbal stalls and stores in the same area were visited. It was found that traditional medicine is not herbal medicine. The assumption that natural medicines are inherently safe is a fallacy. Reliable information is lacking as to their adverse reactions, contraindications and interactions with conventional medicines.

Traditional medicine is a holistic approach to health and illness based on the African world view. It should neither be romanticized nor rejected as charlatanry. Only part of traditional practice is based on natural materials. It this part that should be scientifically validated, documented and promoted. Modern scientific researchers should cooperate in their studies with the traditional healer while in the process protecting the latter's intellectual property rights. Through this approach it is hoped that new sources will be detected for safe, effective and affordable remedies.

Simple and cost-effective liquid chromatographic method for determination of pyrimethamine in whole blood samples dried on filter paper

Minzi OMS, Massele AY, Gustafsson LL, Ericsson Ö.

Department of Clinical Pharmacology, Faculty of Medicine, PO BOX 65010, Dar Es Salaam Tanzania.

A cost effective HPLC method for determination of pyrimethamine (PYR) in human whole blood samples dried on filter paper (Whatman) is reported.

Objective: To develop a filter paper method for determination of PYR in whole blood dried on filter paper

Method: Whole blood spiked with PYR was transferred (100µl) onto filter paper and dried at room temperature. Trimethoprim (TMP) was used as an internal standard. PYR and its internal standard (IS) TMP were extracted into di-isopropyl ether as bases and then re-extracted with 150 µl mobile phase**.** A C-18 column was used and the mobile phase consisted of phosphate buffer (0.05M, pH 5): acetonitrile: concentrated perchloric acid, (750:300:2.5, v/v). Capillary blood samples (100µl) after ingestion of 3 tablets of sulfadoxine-pyrimethamine (SP) by one subject were also tested.

The absorbances of PYR and IS were monitored at 270 nm.

Results: The limit of quantification was 40 ng/ml. The within assay and between assay coefficient of variations were <10% at the limit of quantification.

Conclusion: A filter paper method for determination of PYR in whole blood using small volumes of blood samples has been developed. The method is field adapted and is suitable for application s in laboratories of resource limited countries.

A comparison of different humate preparations: Chemical fingerprinting and anti-inflammatory activity

T.W. Mokoele, A.D. Cromarty, C.E.J. van Rensburg

Department of Pharmacology, University of Pretoria, P.O. Box 2034, Pretoria, 0001 South Africa (e-mail:s2339423@tuks.co.za)

This is the first study to investigate both the anti-inflammatory effects and chemical characteristics of humic acid products from the following sources and geographical locations; four products derived from brown coal (Australia, China, Germany, USA), a bituminous coal (SA), sapropel (Russia), shilagit (India) and a synthetic humate.

Thermogravimetric analysis and TLC were used to fingerprint and compare the various humic acid samples to identify possible chemical and structural differences. Thermogravimetry revealed significant differences in terms of the volatile compounds in most of the samples whereas TLC showed almost no differences with most of the samples except sapropel and shilagit.

The expression of CR3 adhesion molecules on activated neutrophils is associated with the initial phase of inflammation. The effect of the various humic acids on the expression of CR3 by resting and PMA-stimulated neutrophils was investigated flow cytometrically. All samples suppressed CR3 expression at 100μg/ml, but only sapropel was still active at a concentration of 50μg/ml.

The cytotoxicity of the various humic acid samples was determined *in vitro* using human lymphocytes and the MTT assay. None of the humic acid products were toxic to lymphocytes up to a concentration of 1mg/ml.

The lack of cytotoxicity together with the decrease in CR3 expression makes sapropel an ideal candidate for further evaluation in animal models of inflammation.

Local understanding, perceptions and reported practices of mothers / guardians and health workers on childhood malaria in a Tanzanian district - implications for malaria control

C. Comoro[1], S. E. D. Nsimba[2], M. Warsame[3] and G. Tomson[4]

Department of Sociology, University of Dar-es-Salaam, Tanzania[1], Department of Clinical Pharmacology, Muhimbili University College of Health Sciences, Dar-es- Salaam, Tanzania[2], Division of International Health (IHCAR), Department of Public Health Sciences[3] and THE Medical Management Centre[4], Karolinska Institute, Stockholm, Sweden. Email: snsimba@muchs.ac.tz

Knowledge on local understanding, perceptions and practices of care providers regarding management of childhood malaria are needed for better malaria control in urban, peri-urban and rural communities. Mothers of underfive children attending five purposively selected public health facilities in the Kibaha district, Tanzania, were invited to participate in ten focus group discussions (FGDs). The health workers of these facilities were included in six other FGDs to elicit their professional views. Analysis was done using interpretative and qualitative approaches.

Both health workers and all mothers were clear about the signs and symptoms of *homa ya malaria*, a description consistent with the biomedical definition of mild malaria. Although most of the mothers related this to mosquito bites, some did not. Mothers also described a severe childhood illness called *degedege*, consistent with convulsions. Most of the mothers failed to associate this condition with malaria, believing it is caused by evil spirits. Urinating on or fuming the child suffering from *degedege* with elephant dung were perceived to be effective remedies while injections were considered fatal for such condition. Traditional healers were seen as the primary source of treatment outside homes for this condition and grandmothers and mother in-laws are the key decision makers in the management.

Our findings revealed major gaps in managing severe malaria in the study communities. Interventions addressing these gaps and targeting mothers/guardians, mother in-laws, grandmothers and traditional healers are needed.

Hypothalamic-pituitary-adrenal axis function and hypothalamic-pituitary-thyroid axis function in mentally retarded patients with and without self-injurious and/or aggressive behaviour

P.M. van Zyl, *C.A. Gagiano, **C.J. Bester, A. Walubo

Department of Pharmacology, *Westdene Research Centre, and **Department of Biostatistics, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

Introduction and aim*:* The etiology of aggression and self-injuring behaviour in low functioning mentally retarded patients is multi-factorial and may reflect the presence of undiagnosed psychiatric conditions, unapparent due to the degree of the patient's impairment. The study investigates the possibility that inappropriate expression of aggression in low functioning mentally retarded patients may represent an abnormal neuro-endocrine stress response in such patients, as reflected in abnormal hypothalamic–pituitary-adrenal (H-P-A) axis function and abnormal hypothalamic-pituitary-thyroid (H-P-T) axis function.

Patients and methods*:* The H-P-A axis function and H-P-T axis function were compared in adult institutionalised mentally retarded patients with and without self-injuring and/or aggressive behaviour. The groups were matched for age, gender and level of functioning. A behaviour profile was compiled for each of the participants and the dexamethasone suppression test as adapted by Caroll and the thyroid-releasing hormone stimulation test were performed.

Results*:* The results of 44 subjects were included in the final analysis. Baseline hypercortisolaemia occurred in 22,7% of the aggressive subjects and in 9,1% of the non-aggressive subjects. Cortisol non-suppression was demonstrated in 9,1% in the aggressive group and 4,5% of the non-aggressive group. There was a tendency towards a higher baseline cortisol in the aggressive group. Subjects with more recent aggressive activity showed higher baseline cortisol levels. Two male subjects in the aggressive group showed a blunted TRHST combined with DST non-suppression. An exaggerated response was demonstrated in 13.6% of the non-aggressive group and 4.5% of the aggressive group.

Conclusion*:* The findings of the study do not support a role for HPA/HPT axis function in the etiology of aggression in this population. However, the small sample size and the non-homogenous group could have had a major impact on the findings. Longitudinal studies are needed to evaluate HPA axis function in unmedicated patients, in addition to comparing HPA axis function during different kinds of treatment.

Early rescue surfactant treatment for respiratory distress syndrome in preterm lambs: Comparison of a novel synthetic surfactant with bovine-derived surfactant.

J.M. van Zyl[1], J. Smith[1,2], D.M. Barry[1], A. Hawtrey[1], J.J van Rensburg[2], G. Maritz[3]

University of Stellenbosch[1] and Tygerberg Hospital[2] and the University of the Western Cape[3].

Adult animal models of surfactant depletion are not fully representative of the neonatal respiratory distress syndrome (RDS). In the preterm lamb, RDS closely resembles that of the human infant with the advantages being the ability to measure multiple variables for the lungs and other systems over extended periods.

Objective of the study was to investigate if a polymer containing surfactant (PCS) leads to improved systemic oxygenation and lung mechanics in comparison to a commercially available bovine-derived lung surfactant (Survanta®).

A caesarean section was performed on time-dated pregnant Dorper ewes. Premature animals were delivered, sedated and paralysed. Ventilation was started and held constant throughout the study. Lambs were assigned into one of two groups (n=6 lambs/group) within 30 minutes of delivery.

Arterial blood gases, ventilator indices and haemodynamic variables were similar for both groups for the 5-hour study period. Both groups experienced significant deterioration in systemic oxygenation over the study period.

The efficacy (or lack thereof) of early rescue treatment of RDS in preterm lambs with PCS or Survanta® is disappointing, yet in agreement with that of others. The progressive deterioration in systemic oxygenation is probably due to rapidly developing ventilation-perfusion mismatch, due to surfactant inhibition caused by protein-fluid leakage into the airways of the preterm animals.

Investigation of the neuroprotective effects of 17β- Estradiol and Progesterone in the N-acetylhomocysteine-induced neurodegenerative model

Wing Man Wu, Deepa Maharaj, Himant Maharaj and Santy Daya

Faculty of Pharmacy, Rhodes University, Grahamstown, 6140, South Africa (E-mail: s.daya@ru.ac.za)

An elevated plasma level of the Homocysteine (Hcy) is now recognized as an independent risk factor for dementia and AD. Several studies have shown increased plasma Hcy levels in healthy elderly people, which could increase the risk of suffering degenerative disease by the impairment of redox processes and the increase of oxygen-free racicals during aging. Evidence show that Hormones Replacement Therapy (HRT) preferentially protects against a decline in verbal memory in healthy postmenopausal women and decreases the risk of AD. Numerous mechanisms have been identified whereby Estrogen and Progesterone affects structures and functions in the brain that are implicated in explicit memory. The neuroprotective effects of Estrogen and Progesterone (Pre- and Post-treatment) in homocysteine-induced neurodegeneration effects in the hippocampus *in vivo* were investigated. Treatment with either Estrogen or Progesterone alone showed a significant decrease in Hcy-induced superoxide anion generation and lipid peroxidation. Moreover, using histology and apoptosis testing shows that Estrogen or Progesterone alone is able to protect the hippocampal neurons against Hcy-induced neuronal damage and programmed cell death. Furthermore, Estrogen and Progesterone show antioxidant effects by increasing the antioxidants such as GPx, SOD and catalases in Hcy-induced neuronal damage. Thus, showing the effectiveness of Estrogen and Progesterone against Hcy-induced neurodegeneration.

3 Young Scientist Presenters

Nitric oxide involvement in an animal model of post-traumatic stress disorder (PTSD)

T Bothma, A Nel, D.J. Stein[1] and B.H. Harvey
Departments of Pharmacology and [1]Psychiatry, North-West University and [1]University of Stellenbosch (e-mail: fklbhh@puk.ac.za).

PTSD is associated with shrinkage of the hippocampus that may have its origin in stress-induced excitotoxicity. Animal studies have highlighted a potential role for glutamate and nitric oxide (NO) in the stress response[1].

Study Objectives: Using an animal model of repeated trauma[2], we investigated the effect of stress on hippocampal release of nitrogen oxides (NO_x) and its dependency on glutamate, NO and cGMP activity using drugs selective for these pathways.

Summary of Methods: Rats were exposed to stress[2] together with saline or drug administration during and up to one week post-stress. Rats were sacrificed for assay of hippocampal NO_x. Animals also received either the glutamate NMDA receptor antagonist, memantine (MEM;5mg/kg ip/d), the NO synthase inhibitor, 7-nitroindazole (7-NI;20mg/kg ip/d), the cGMP-specific PDE inhibitor, sildenafil (SIL;10mg/kg ip/d) or the NFκ-β antagonist, pyrollidine dithiocarbamate (PDTC;70mg/kg ip/d).

Results: Stress significantly increased hippocampal NO_x. This increase was blocked by pre-treatment with either PDTC or 7-NI, while MEM was without effect. SIL significantly augmented stress-induced NO_x accumulation.

Conclusions: Stress activates the NO-cGMP pathway, possibly involving both nNOS and iNOS. Sub-cellular NO-modulation may represent a therapeutic strategy in preventing the effects of severe stress. The value of NMDA receptor antagonism, however, appears limited.

References: [1]Harvey et al (2004). *Psychopharmacol (Berl). in press*; [2]Harvey et al (2003) *Brain Res* 983: 97-107.

An investigation into the possible neuroprotective effects of the curcuminoids against lead (Pb)–induced neurotoxicity in primary hippocampal neurons.

Dairam, G.M. Watkins and S. Daya
Faculty of Pharmacy, Rhodes University, Grahamstown, P.O. Box 94, 6140 South Africa (e-mail: s.daya@ru.ac.za)

Introduction: Alzheimer's disease is a neurodegenerative disorder characterised by loss of memory, learning ability and the reduction in ability to perform basic activities. One of the major causes, free radical damage to neuronal tissue, has shown to be reduced by anti-oxidants. Turmeric is a yellow coloured compound found in the rhizomes of *Curcuma Longa*. Three major phenolic compounds have been isolated from turmeric namely curcumin, demethoxycurcumin and bisdemethoxycurcumin and has been known since ancient times to possess medicinal activity. In addition to its powerful anti–oxidant activity, it also has anti–inflammatory properties, HIV antiproteases activity and cancer preventative properties. Lead, a widely used metal that is present in the environment causes widespread tissue damage and antagonises many biological actions of Ca^{2+} ions. Our past results have shown that Pb– induced lipid peroxidation in rat brain homogenate was significantly reduced by the co–treatment of the homogenate with turmeric.

Objectives: It was therefore of interest to determine whether the individual components of turmeric could reduce Pb –induced neuronal damage in primary hippocampal neurons.

Methods: HPLC–MS and TLC were used to determine the presence of the three curcuminoids in turmeric. The curcuminoids were separated from turmeric using a semi–preparative HPLC method. An isocratic mobile phase (37% acetonitrile) was used with a Phenomenex C18 RP semi–preparative column and guard column. Primary hippocampal neurons were established from 3–4-day-old rat pups. Experiments were performed using 7-day-old cultures.

Results: The three curcuminoids that were present in the turmeric were successfully separated. NMR and TLC confirmed the purity of the extracts. Pb-induced neuronal damage was significantly reduced by the co-treatment of the neurons with the curcuminoids.

Neuroprotective properties of sildenafil and its modulating effects on muscarinic acetylcholine receptor function.

B.E.Eagar, C.B.Brink, and B.H.Harvey
Division of Pharmacology, North-West University (PUK), Potchefstroom, South Africa (e-mail: fklcbb@puk.ac.za).

Sildenafil, a selective phosphodiesterase-5 (PDE5) inhibitor, is indicated for the treatment of male erectile dysfunction. However, a role in neuroprotection has been suggested, possibly linked to its action on the NO/cGMP pathway, which has been implicated in the aetiology of depression and anxiety.

The aims of the study were to investigate any neuroprotective properties of sildenafil, as well as modulating effects of sildenafil pre-treatment on muscarinic acetylcholine receptor (mAChR) function.

Human neuroblastoma SH-SY5Y cells were seeded in 24-well plates and pre treated in serum-free medium (oxidative stress) with no drug (control), sildenafil (100nM and 450 nM), dipiridamole (20 µM), zaprinast (20 µM), 3-isobutyl-1-methylxanthine (IBMX - 1mM), N^2,2'-O-dibutyrylguanosine 3',5'-cyclic monophosphate sodium salt (500 µM), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ – 3 µM) or sildenafil + ODQ (450 nM and 3 µM respectively). Thereafter cells were radiolabelled with 1 µCi/ml [^{3}H]-*myo*-inositol or dummy-labelled and dose-response curves of methacholine (measuring [^{3}H]-IP_x accumulation) constructed or the trypan blue and MTT tests for cell viability applied.

Sildenafil pre-treatments caused a 2.5-fold increase in the E_{max} value of the methacholine. The trypan blue test suggests that the PDE5 inhibitors and cGMP analogue protect cells against oxidative stress. The MTT test was not suitable, since pre-treatment with the abovementioned drugs inhibited the formation of formazan.

Sildenafil up-regulates mAChR function in SH-SY5Y cells and displays neuroprotective properties in these cells, likely to be associated with its PDE5 inhibitory action.

Characterization of the α2A-lytic effects of mirtazapine and its effects on muscarinic acetylcholine and β-adrenergic receptor functions

K. Khoza*, C.B. Brink*, and B.H. Harvey*
*Division of Pharmacology, North-West University (PUK), South Africa (email: fklkk@puknet.puk.ac.za).

Mirtazapine is an atypical antidepressant with α_2-adrenoceptor (α_2-AR) lytic action, thought to play a role in its putative earlier onset of action. However, it is not known whether mirtazapine is a neutral antagonist or inverse agonist at α_2-ARs.

This study aimed to determine the mode of α_2-AR antagonism by mirtazapine, as well as to investigate its modulatory effects on β-AR and muscarinic acetylcholine receptor (mAChRs) function.

Chinese hamster ovary (CHO-K1) cells expressing the porcine α_{2A}-AR at high levels (α_{2A}-H), its constitutively active mutant (α_{2A}-CAM), or mock-transfected controls (neo), were radiolabelled with [^{3}H]-adenine and dose-response curves of mirtazapine, yohimbine, mianserin or idazoxan constructed, measuring [^{3}H]-cAMP accumulation. Human neuroblastoma (SH-SY5Y) cells were pre-treated with 0 or 10 µM mirtazapine for 24 hours, followed by washing and radiolabelling with [^{3}H]-*myo*-inositol or [^{3}H]-adenine. Thereafter, respectively, dose-response curves of methacholine-was constructed (measuring [^{3}H]-IP_x accumulation) to investigate mAChR function, or *l*-isproterenol-induced responses ([^{3}H]-cAMP accumulation) was measured to investigate β-AR function.

Mirtazapine did not affect [^{3}H]-cAMP accumulation in α_2-H or α_2-CAM cells, but displayed partial inverse agonism in α_2- CAM cells. Mirtazapine pre-treatment in SH-SY5Y cells did not alter muscarinic receptor function, but reduced *l*-isoproterenol-induced increase in [^{3}H]-cAMP accumulation in SH-SY5Y cells.

In conclusion, mirtazapine is a partial inverse agonist at the α_2-ARs. In contrast to fluoxetine and imipramine it does not modulate mAChR function. It reduces β-AR function.

Characterization of the situational reminder in the rodent Time Dependent Sensitization (TDS) Model of Post Traumatic Stress Disorder

Z.I. Jeeva*, L. Brand*, B.H.H. Harvey*
*Division of Pharmacology, North-West University (Potchefstroom Campus), RSA. (e-mail: fklzj@puknet.puk.ac.za).

This study aimed to establish the necessity and to characterize the effect of the "re-stress"(RS) session as a situational reminder in a previously validated TDS model, using plasma corticosterone (CORT) levels and monoamine concentrations the hippocampus, as markers.

Forty rats were exposed to repeated trauma (2 hr restraint stress, 20 minute swim stress and exposure to 0.8ml of 4% halothane gas till loss of consciousness) where after: Group 1: sacrificed immediately after repeated trauma, Group 2: sacrificed 7 days thereafter, Group 3: sacrificed on day 7, immediately after RS.(20 min swim stress); Group 4:sacrificed on day 14, 7 days after RS. Group C was included as a non-stressed control. Data was analyzed using a Dunnett's *t*- test, $p<0.001$ (*n=10).*

CORT levels, (determined by radiometric analysis), increased significantly in Gr 1 vs Gr C and Gr 3 vs Gr C exhibited a significant reduction. Monoamine determination using electrochemical HPLC showed 5-HT concentrations decreased in Gr 2 vs Gr C. Gr 3 had increased NA and DA concentrations vs Gr C, while Gr 4 had decreased NA and 5-HT concentration.

PTSD may be associated with hypocortisolemia. RS caused a decrease in 5-HT and CORT concentrations but increased NA and DA concentrations, it was concluded that RS is necessary for the development and progression of stress-related pathology of PTSD.

Aspirin and Acetaminophen Protects Against 1-Methyl-4-Phenylpyridinium-Induced Mitochondrial Toxicity

H. Maharaj, D.S. Maharaj, W.M. Wu and S.Daya
Division of Pharmacology, Department of Pharmacy, Rhodes University, Grahamstown, 6140,South Africa.

The cellular damage caused by MPP^+ is primarily the result of the mitochondrial respiratory inhibition at the level of complex I. We therefore assessed the effect of non-narcotic analgesics, acetaminophen and aspirin on complex I and electron transport chain activity *in vivo* in rats treated intranigrally with 1-methyl-4-phenyl pyridinium (MPP^+). Intranigral infusion of MPP^+ in rats caused severe inhibition of mitochondrial complex I activity and a decrease in electron transport chain activity. Systemic post-treatment of rats with aspirin, acetaminophen and the combination of aspirin and acetaminophen prevents the degenerative effects of MPP^+ in the mitochondria i.e. complex I and electron transport chain activity. While these findings suggest usefulness of non-narcotic analgesics in neuroprotective therapy in neurodegenerative diseases, aspirin appears to be a potential candidate in prophylactic as well as in adjuvant therapy in Parkinson's disease.

The Effect Of Melatonin, 6-OHM and QA on the Level of hsp-70 in the Rat Hippocampus

D.S. Maharaj[1], T. Matambo[2], G. Blatch[2], B.D Glass[3], and S. Daya[1*]
[1]Division of Pharmacology, Faculty of Pharmacy, [2]Department of Biochemistry, Microbiology, and Biotechnology, Rhodes University, Grahamstown, South Africa. [3]School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Australia.

The hippocampus is involved in memory formation and thus any damage to these neurons would have deleterious effects on learning and memory and could lead to various types of dementia. In view of the previous experiments, which have shown that 6-OHM protects against QA-induced neuronal damage, the present study was performed to investigate a further possible mechanism for this protection at the molecular level. Melatonin has been shown to protect against QA-induced oxidative neurotoxicity. However to date no work has been done on the effect of melatonin on Hsp70 expression. Thus, it was decided to analyze the level of Hsp70 expression in hippocampal neurons, in response to treatment with QA alone, MEL alone, 6-OHM alone and the combination of each drug with QA using Western analysis. The results of the western blotting show that the level of Hsp70 in the hippocampus increases in response to treatment with QA. This was expected since Hsp70 is expressed when the cell is under stress such as that resulting from free radical attack, which is initiated by QA-induced damage. Melatonin shows to be protective to hippocampal neurons by itself and in combination with QA as it decreases the rise in Hsp70 expression caused by QA. However, 6-OHM proved to induce hsp 70 expression.

It may be possible that 6-OHM induces the expression of both forms of Hsp70, but that the level of Hsc70 that is expressed in response to 6-OHM is much higher than the inducible form. The reason for this rationale stems from the fact, that although 6-OHM is implicated as a prooxidant, it is also a potent neuroprotectant, and thus if it was causing stress to such an extent then one wouldn't observe the protective effects against agents such as QA and the results seen in this paper would then represent that for the inducible Hsp70. Another possibility is the fact that Hsp70 induction may contribute to the protective effects of 6-OHM as Hsp's protect neural cells from subsequent stress that would normally be damaging or lethal.

Cardiovascular Effects of the Aqueous Extract of Leonotis Leonurus in Normotensive Rats

K.C.Obikeze[1], P. Mugabo[1]
Department of Pharmacology[1], University of the Western Cape, Private Bag X17, Bellville, 7535. South Africa. (e-mail: pmugabo@uwc.ac.za)

Leonotis leonurus (L.L.) is used in traditional medicine for treatment of various ailments including hypertension[1]. An aqueous extract of the leaves and stems of LL was found with a positive inotropic and a negative chronotropic effect on the isolated perfused rat heart[2].

This study was aimed at determining the cardiovascular effects of the aqueous extract of L.L.leaves on male wistar rats (250-350g). They were anaesthetized with sodium pentobarbitone (40mg/kg) administered by IP route. After tracheotomy, the external jugular vein was cannulated for infusion of test substances. The femoral artery was cannulated and connected via a pressure transducer to the PowerLab 4/20T for recording of the systolic (SP), diastolic (DP) and mean arterial pressures (MAP) and the heart rate (HR).

L.L. and control agents (adrenaline and atenolol) were dissolved in normal saline and administered at a rate of 0.1 ml/min. until the maximum effect was obtained (3 min.).

Results were expressed as a difference between the base line preceding the administration of each tested substance and at steady state. They were analyzed using the Student's t-test. Differences between two related means were considered statistically significant for P values equal or less than 0.05.

L.L. (0.5 to 7mg) significantly (p <0.05) increased the SP (4 ± 0.4 to 17.2 ± 1.1 mmHg), DP (-1.7 ± 0.7 to 12.1 ± 4.2 mmHg) and MAP (2.1 ± 0.5 to 15.5 ± 2.1mmHg) and decreased the HR (23.5 ± 5.78 to -20.0 ± 2.88 bpm). Atenolol (2mg) significantly ($p<0.05$) decreased the effect of LL on the systolic and diastolic pressures. However, the effects of LL on the HR was significantly ($p<0.05$) potentiated by the pretreatment with atenolol.

REFERENCES:

1. Hutchings, A. 1996. *Zulu medicinal plants*. Natal University Press, Pietermaritzburg.

2. Khan, F., Mugabo, P., Burger, A.P. 2001. *Effects of Leonotis leonurus on the isolated perfused rats heart*. Presented at the international immunopharmacology congress. September 2001. Sun City, South Africa.

An investigation into the potential neurotoxic or neuroprotective effects of Metrifonate

Ramsunder, S.Daya
Department of Pharmacology, Faculty of Pharmacy, Rhodes University, Grahamstown, 6140, South Africa. s.daya@ru.ac.za.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, in which there is a marked decline in neurotransmitters, especially those of the cholinergic pathways One of the drugs investigated for the symptomatic treatment of this disease is Metrifonate. Metrifonate is an organophosphate compound used as an acetylcholine esterase inhibitor. It is a non-active pro-drug which is non-enzymatically converted, into its active metabolite, Dichlorvos (DDVP), which inhibits both Acetylcholine esterases, and Butylcholine esterases. Although Phase 3 clinical trails were supportive of the use of this drug in the symptomatic treatment of Alzheimer's disease, it's FDA application was withdrawn, due to concern's of reversible but clinically significant muscle weakness and respiratory depression. A drug used in a neurodegenerative disorder, should preferably not induce further neurotoxicity. This sparked our interest and further investigation into the possible neuroprotective or neurodegenerative properties of this drug.

In assessing the neuroprotective properties of this drug, brains from untreated animals were homogenised and exposed to varying concentrations of metrifonate. In the in vivo study male adult rats were treated for five days with varying doses of Metrifonate. The results show that the drug significantly increases lipid peroxidation in both experiments. These results imply that metrifonate is neurotoxic in that it induces lipid peroxidation by increasing oxidative stress.

An in vitro investigation to measure the activity of the respiratory chain was determined spectrophotometrically at 600nm using a modified technique described by Plummer et al. The activity was determined by the the change in absorbance by the reduction of a synthetic dye, 2,6-dichlorophenolindophenol (used as an electron acceptor) in the presence of l-malate. Rat brain homogenate was first exposed to varying concentrations of Metrifonate, and the biological activity was measured by the change in absorbance. The results indicated a decrease in biological oxidative function, thus indicating that Metrifonate may have the potential to disrupt the electron transport chain and impair cellular functions.

These results imply that metrifonate has the potential to be neurotoxic in that it induces lipid peroxidation by increasing oxidative stress and disrupting the biological oxidation of the cell at the level of complex one. Thus drugs such as metrifonate should be used with caution in neurodegenerative disorders such as Alzheimer's disease.

Loss of antidepressant efficacy after imipramine discontinuation is associated with nitric oxide synthase (NOS) activation.

R Retief, A Nel and B.H. Harvey
Department of Pharmacology, North-West University, Potchefstroom (e-mail: fklbhh@puk.ac.za).

Inappropriate antidepressant discontinuation invokes long-term morbidity, and appears linked to hippocampal shrinkage[1]. Depression has been associated with disturbances in excitotoxic glutamate-nitric oxide (NO) activity[2].

Study Objectives: Imipramine (IMI) treatment and withdrawal was studied using a learned helplessness paradigm[3]. Behavioural changes and hippocampal NOS activity was determined together with pharmacological manipulation of the glutamate-NO-cGMP pathway.

Summary of Methods: Rats received either saline or IMI (15mg/kg/d ip) for 3 weeks, followed by acute withdrawal for 7 days, whereupon animals received either saline, the NMDA receptor antagonist, memantine (MEM;5mg/kg/d ip) or the NOS/guanylyl cyclase inhibitor, methylene blue (MB;15mg/kg/d ip). Swim stress-induced immobility and locomotor behaviour were determined after IMI treatment and after withdrawal, followed by radiometric assay of hippocampal NOS.

Results: IMI significantly decreased immobility time and reduced NOS activity. IMI withdrawal increased swim stress and profoundly increased NOS. MEM and MB re-established the anti-immobility effects and reversed NOS hyper-function during IMI withdrawal, without themselves effecting swim mobility. MB altered locomotor activity, while both drugs alone inhibited NOS.

Conclusion: Loss of antidepressant efficacy is associated with NOS activation, reversible by blocking the glutamate-NOS pathway. Increased NO after inappropriate withdrawal may mediate neuro-degenerative pathology associated with poor compliance and treatment resistance.

References: [1]MacQueen et al (2003). *PNAS* 100: 1387-1392; [2]Harvey et al (2003). *Biol Psychiatry* 54: 1105-1117; [3]Harvey et al (2002). *Life Sci* 71: 45-57.

4 Poster Presentations

Cognitive dysfunction and serotonin receptor changes evoked by stress-restress are modified by ketoconazole

Brand L*, Harvey B.H.*, Naciti C*, Stein D.J#.

*Division of Pharmacology, North-West University, South Africa, #University of Stellenbosch, South Africa. email: fkllb@puk.ac.za

Structural changes in the hippocampus and the medial prefrontal cortex in post-traumatic stress disorder (PTSD), appear correlated with illness severity and cognitive deficits. Elevations in circulating glucocorticoids have been implicated in this process, possibly impacting on the serotonin (5-HT) system so contributing to other symptoms. Although increased plasma cortisol levels occur immediately after or during stress, suppression of plasma cortisol characterises PTSD.

This study's objective was to investigate the effect of inhibition of adrenal steroid synthesis on the cognitive dysfunction and serotonin receptor changes evoked by the stress-restress procedure previously found.

Male Sprague-Dawley rats were exposed to sequential stressors viz. restraint stress, forced swim stress, and finally exposure to halothane vapours which was followed 7 days later with a brief re-stress procedure. Spatial memory was studied using a Morris water maze (MWM) procedure. Receptor binding analyses were done to determine receptor density (Bmax) and affinity (Kd) values for 5-HT_{1A}-receptors in the hippocampus and 5-HT_{2A} –receptors in the frontal cortices. Ketoconazole (KCZ) 24mg/kg/d x 2 weeks ip treatment was started one week before stress and continued throughout the TDS procedure.

On day 7 post-stress, significant spatial memory impairment, increased $5HT_{1A}$ receptor density and decreased $5HT_{1A}$ receptor affinity, and increased $5HT_{2A}$ receptor affinity was observed (Dunnett s t-test, $p< 0.05$). KCZ prevented the deleterious effects of stress on cognitive functioning and reversed the hippocampal $5HT_{1A}$ receptor affinity changes as well as the stress effects on $5HT_{2A}$ receptor affinity.

The data described here as well as clinical studies support the possible clinical use of KCZ in treating PTSD.

Declaration: This data was presented as a poster at the CINP congress in Paris (20 – 24 June 2004).

Direct compression of a novel intra-ocular poly(DL-lactide-co-glycolide) (PLGA) implant: Investigation of the micromeritic parameters

Y.E. Choonara, V. Pillay and M.P. Danckwerts*

Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Rd. Parktown 2193, Johannesburg, South Africa (*correspondance: danckwertsmp@therapy.wits.ac.za).

The aim of this study was to investigate the suitability and effect of the micromeritic properties on the flowability of PLGA used in the formulation of a novel doughnut-shaped minitablet (DSMT) implant, employing direct compression.

Particle size, shape, and flowability of various viscosity grades of PLGA (Resomer®) were studied. The rugosity of particles was characterized by scanning electron microscopy (Jeol-840 SEM). Flowability was evaluated using the angle of repose (*è*) method. Particle size was analyzed via a frequency distribution polygon. The geometric mean sizes (*M*) and geometric standard deviations ($ó_g$) were determined from cumulative distribution curves and other statistical descriptors.

SEM images revealed different particle geometry, which contributed to variable flowability (*è* = 28.98 ± 3.11). An increase in the geometric mean diameter provided a decrease in the angle of repose. Size analysis showed positively skewed distributions (*IQCS* = +0.411) with 71% of particles falling in the mean size range of 169-356 µm for Resomer® RG502, RG503 and RG504. These size distributions were regarded as pharmaceutically tight ($ó_g$ = 1.764).

In conclusion the micromeritic properties of PLGA significantly influenced its flowability and packing, making it suitable for direct compression.

A Retrospective Comparative Study of Therapeutic Drug Monitoring Parameters with Pharmacokinetic-Pharmacodynamic Parameters of Gentamicin in Some Patients at Universitas Hospital.

MF van Jaarsveld and Walubo, A

Department of Pharmacology, University of the Free State, South Africa.

Introduction and aim: Although it is well understood that pharmacokinetic-pharmacodynamic (PK/PD) parameters which integrate pharmacokinetics and antimicrobial activity are a powerful aid to the selection of antibiotics for specific organisms as well as to predicting outcome of treatment, this concept has not been yet adopted in many institutions. In these institutions, the use of antibiotics such as gentamicin is still optimised by serum concentration monitoring (i.e., therapeutic drug monitoring, TDM) which is mainly addressed to minimising toxic effects without revealing anything about antimicrobial activity. However, since TDM involves measurement of peak and trough gentamicin plasma concentrations, it was decided to undertake a three year (2001-2003) retrospective study of patients on gentamicin in Universitas hospital on whom TDM was done, to determine whether the required PK/PD parameters such as the peak/minimum inhibitory concentration (MIC) ratio, were achieved.

Methods: Of the 88 records of patients who used gentamicin, only 59 met the criteria for inclusion. Of these, culture and sensitivity (C/S) was positive in 20, negative in 30 and not done in 9. Of the 30, eight had received gentamicin empirically for infective endocarditis. The organisms in the 20 comprised of: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanni* and *Proteus mirabilis*. Evaluation for the Peak/MIC ratio, was done separately for each organism and calculations were done for both the lower and higher MICs.
Results: The required TDM parameters of gentamicin were achieved in most patients (trough < 2 mg/L and peak > 8mg/L). Using the lower MIC, the Peak/MIC ratios were as follows: 19.6±8 for *Escherichia coli*, 125±7 for *Klebsiella pneumoniae*, 9±3 for *Pseudomonas aeruginosa*, >1 for *Acinetobacter baumanni* and 4.6±1.4 for *Proteus mirabilis*. Only the last two organisms did not achieve the required Peak/MIC ratio of greater than 8. For the higher MIC, the Peak/MIC ratios were far below the required value.

Conclusion: The TDM and PK/PD parameters appeared to agree with each other when the lower MIC was used for most organisms, which implies that the two methods can be use together to prevent toxicity (TDM) and to determine therapeutic outcome (PK/PD).

Adding a mucolyticum to the treatment of sinusitis does not have an effect on the healing rate.

JP Meeding, JR Snyman.

Department of Pharmacology, University of Pretoria, PO Box 2034, Pretoria, 0001 South Africa (e-mail: ronel.meeding@up.ac.za).

The aim of the study was to establish whether the use of a mucolyticum has any effect on the healing rate of acute sinusitis over and above that of the antibiotic therapy.

40 Patients suffering from acute sinusitis, were randomly divided into 2 groups.

One group was treated with amoxicillin and a carbocysteine suspension and the second group was treated with amoxicillin and a placebo suspension. They were evaluated according to a scoring system for symptoms after 10 days.

No statistically significant difference was observed in the healing rate between the 2 groups after 10 days.

In conclusion, the addition of a mucolyticum in the treatment of patients with acute sinusitis could not be proved to have a beneficial effect.

Table I. Comparison of the 2 groups after 10 days

Symptoms after 10 days:	**Group C**	**Group P**	**p-value**
Headache Cleared:	16	16	1.000
Facial pain Cleared:	16	14	0.635
Postnasal drip Cleared:	8	6	0.701
Cough cleared:	16	13	0.882

1. Desrosiers M., Frenkiel S., et al. Acute bacterial sinusitis in adults: Management in the Primary Care setting. The Journal of Otolaryngology, nr 2, October 2002: 2S2-2S 13.

2. Hickner John M., Bartlett John G, et al. Principles of appropriate antibiotic use for acute rhinosinusitis in adults: background. Ann Intern Med. 2001;134: 498-505.

Solid-Phase Extraction (Spe) and Reverse-Phase High Performance Liquid Chromatography (Rp-Hplc) of Combretum Woodii Leaf.

M E Mothibe[1a]; J P H van Wyk[a]; J N Eloff[b]

[1] School of Pharmacy; [a]Department of Pharmacology & Therapeutics, Medunsa; [b]University of Pretoria

OBJECTIVE: *Combretum woodii* is a tree belonging to the family C*ombretaceae*, of the subgenus *Combretum*. Many species of this family are widely spread in Southern Africa and are used for a variety of conditions including pneumonia, syphilis, colds, chest cough, diarrhoea, heartburn, headache and as a lucky charm. Leaf extracts of some of the Southern African members of the *Combretaceae*, including *Combretum woodii* have been found to have antibacterial activity against *Staphylococcus aureus, Eschericia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*. The aim is to develop a rapid and effective method of extraction and isolation of the plant compounds present in the leaf, and to show the complexity of each extraction.

METHODS: Milled leaf material of *Combretum woodii* was extracted with an acetone:water (10:1) solution on a shaker. The preliminary extract was extracted by solid phase using bonded-silica SPE tubes (Supelclean™ LC 18) with solvents of different polarity indeces; methanol:water(65:35), hexane and chloroform respectively. Each of the eluents was filtered (0.45µm) and analysed by reverse-phase chromatography on an HPLC system with a diode array detector, using acetonitrile as the mobile phase.

RESULTS AND DISCUSSION: Analysis of the preliminary acetone extract shows the presence of a variety of compounds that do not separate well on HPLC.

Each of the solvent extracts shows the presence of more than one compound with different retention times. The chloroform extract shows fewer compounds than the hexane and the methanol: water extract.

CONCLUSION: Separation of the plant compounds present in the leaf was achieved and the complexity of each extract was observed. The extracts will be used to examine the effects of the various compounds on human neutrophils, by biochemilenescence technique and the identity of the compounds will be confirmed.

Identification of anti-babesial activity for four ethnoveterinary plants in vitro

V Naidoo, E Zweygarth, JN Eloff, GE Swan

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria,

Private Bag x04, Onderstepoort, 0110, South Africa (email: vinny.naidoo@up.ac.za)

Babesiosis is a tick-borne disease affecting a wide range of vertebrate hosts in both the tropical and sub-tropical parts of the world. Clinically the babesial protozoan parasite is related to malaria by having an almost identical clinical presentation and belonging to the phylum Apicomplexa. Locally there are several plant remedies in use by small-scale rural farmers to treat babesiosis. Their choice in herbal remedies is based solely on knowledge passed down from generation to generation.

A commonly available *Babesia caballi* culture system was utilized to determine if *in vitro* anti-protozoal activity was present. Well-established *B. caballi* cultures, with 250 µl of infected cells, were initially inoculated with either imidocarb diproprionate and diminazene aceturate for the validation of the assay. The degree of inhibition was determined using the colour change within culture flask as well and by the calculating the degree of erythrocyte parasitaemia. The Imidocarb and Diminazene had an EC_{50} value of 0.08 and 0.3 ug/ml respectively. The degree of inhibition as evaluated from the colour change correlated with the mean cell parasitaemia.

Four ethnoveterinary plants, *Rhoiscissus tridentata*, *Elephantorrhiza elephantina*, *Aloe marlothii* and *Urginea sanguinea* acetone extracts were tested following the validation. Only the *E. elephantina* rhizomes extracts was reproducibly effective at a concentration of 100 ug/ml (EC_{50} not calculated). The colour change method of evaluation appeared to be insensitive in determining inhibitory activity of herbal extracts.

Cytotoxicity of polymer-bound methotrexate against Cor L23, a human large cell lung cancer cell line, and its methotrexate resistant subline

Nell, M.J[1]., Neuse, E.W[2]., Meirim, M.G[3]. and Van Rensburg, C.E.J[4].

Department of [1,4] Pharmacology, University of Pretoria P.O.Box 2034, Pretoria 0001. [2,3]Department of Chemistry, University of the Witwatersrand, Johannesburg. margo.nell@med.up.ac.za

Methotrexate represents one of the potent classical antitumor drugs. However, significant toxic side effects and induction of drug resistance continue to restrict its therapeutic effectiveness. A promising synthetic strategy to overcome these deficiencies involves the bioreversible conjugation of the drug with a macromolecular water-soluble carrier. It is postulated that this may change its intracellular kinetics and result in higher potency with fewer systemic side effects.

Tests were conducted on a human large cell lung cancer cell line (Cor L23) and its drug-resistant subline using a series of polyamide-MTX conjugates probing their antiproliferative activity. For comparison, activities were determined against drug sensitive HeLa cells. IC50 values for the Cor L23 cell line, compared to those of the HeLa cell line, reveal activities for the most part exceeding that of free MTX. In the resistant cell line, we found that activities undergo 1ittle change, the resistance factor averaging 0.9. In contrast, a factor of 3.7 is determined for free MTX.

These findings, although preliminary, attest to the inherent pharmacological advantage of MTX conjugation with water-soluble carrier polymers and will provide the basis for more elaborate synthesis and *in vitro/in vivo* screening programs.

Case management of malaria in under-fives at primary health care facilities in a Tanzanian district

Stephen E. D. Nsimba[1,2], Amos Y. Massele[1], Jaran Eriksen[3], Lars Gustafsson[3], Goran Tomson[2] and Marian Warsame[2].

Department of Clinical Pharmacology, Muhimbili University College of Health Sciences, P. O. Box 65010, Dar-es-Salaam, Tanzania[1], Division of International Health (IHCAR), Department of Public Health Sciences, Karolinska Institutet, SE -17176, Stockholm, Sweden[2], and Division of Clinical Pharmacology, Department of Medical Laboratory Sciences & Technology, Huddinge Teaching Hospital, SE-14186, Stockholm, Sweden[3].

Case management of malaria in children under five years of age at primary health care facilities was carried to evaluate the accuracy of self-reported mothers'/guardians' information on chloroquine use in children. A random sample of 652 mothers/guardians with sick children attending 10 primary health care facilities in the Kibaha district were selected. Interviews, observations and blood samples for determination of chloroquine levels and thick smears for detection of malaria parasites were taken from the children who were prescribed chloroquine.

Fever (75%) and respiratory (46%) problems were the most common complaints of the presenting conditions respectively. Fifty four percent of the children reported intake of medications at home, most commonly antipyretics and chloroquine. There was a significantly higher use of antipyretics among home treated children compared to those taken previously to health facilities ($p = <0.001$). Use of antibiotics was higher among children who were taken previously to health facilities ($p <0.0001$).

The average consultation time was 3.8 minutes. Physical examination was performed in 39% of the children with inter-facility variations. Seventy one percent of the children were diagnosed with malaria, as single condition or in combination with

others. Respiratory problems (29%) was the leading over-lapping condition. Malaria parasites were found in 38% of the cases given a malaria diagnosis. Eighty one percent of all prescriptions included analgesics, 71% chloroquine, 54% antibiotics and 24% were of injections.

Of the 529 blood samples successfully analysed for chloroquine, 98% had detectable levels. Ninety-seven percent of the children without history of prior chloroquine treatment had detectable drug levels in the blood, 11% had high levels (≥1000 nmol/L).

There was low quality of care in the facilities in terms of presumptive diagnosis, consultation time, physical examination and prescription of chloroquine. Medication histories by mothers/guardians and care providers need to be improved for better case management of malaria.

Comparative In Vitro and In Vivo Study of a Sugarcoated Chloroquine Preparation Marketed in Tanzania Versus an Ordinary Brand.

Nsimba Sed, Aden-Abdi Y, Rimoy Gh, Massele Ay, Alm C, Ericsson Ö And Gustafsson Ll.

Statement of the problem: Use of sub-standard drugs including sub-therapeutic doses of antimalarials and other drugs is a major problem in most developing countries.

Objective: To investigate the absorption and the quality of a sugar-coated chloroquine (CQ) marketed in Tanzania.

Design, settings and study population: Twenty adult healthy volunteers were randomised to take either the test brand (Group A) or a control chloroquine phosphate (Group B). Each subject received 300 mgs chloroquine base. Whole blood dried on filter papers were collected at time 0 and at 15 and 30 min and at 1, 2, 3, 4, 6, 8, 24, 36, 48, 72 and 168 hrs after drug intake. Urine samples were collected at time 0, 0-4 hrs, 4-8hrs, 8-24hrs, 24-48hrs and 48-72hrs after drug administration. In an in vitro study, six tablets from each of the two CQ preparations were checked for the amount of active drug contained in each tablet and their dissolution rates.

Results: The blood concentration Area Under the Curve (AUC) of group B was about 10% larger than that of group A. The total amounts of CQ plus deethylchloroquine excreted with the urine during the 72hr study period were 5% for group A and 6% for group B. none of the pharmacokinetic parameters were significantly different between the two groups. All the tablets contained the labelled amounts of chloroquine, however, one tablet from the test drug failed to fulfil the required dissolution rate.

Conclusion: We found no major difference between the AUCs of the two CQ preparations, but the sugar-coated brand has shown to have variable dissolution rate. We propose that, there is a need for making quality assessments on regular basis for all locally manufactured and imported antimalarials and other drugs in order to prevent and reduce treatment failures, emergence and spread of drug resistance in the country.

The influence of nutritional supplement on the immune status and haematological parameters of HIV-positive/AIDS patients in the African community of Bloemfontein, Free State, South Africa

O.O Oguntibeju[#], F.E van Schalkwyk* & W.M.J van den Heever[#]

School of [#] Health Technology and * Hospitality and Tourism, Central University of Technology, Free State, P/Bag X20539, Bloemfontein 9300.

The aim of this study was to determine the influence of a nutritional supplement (Africa Solution) on the immune status and haematological parameters of HIV-positive/AIDS patients.

The study population consisted of 35 HIV-positive/AIDS patients from the African community of Bloemfontein. The immune and haematological parameters were determined using standard methods at baseline, during and after supplementation. Results showed that the viral load decreased significantly (P<0.002) with time following supplementation. The mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) increased significantly (P<0.002), reflecting the positive effect of the supplement on reducing the viral load and increasing the haematological parameters. However, the supplement demonstrated no effect on the $CD4^+$T-cell count.

The reduction of the viral load is very important since median survival time is known to increase with reduction in HIV viral load suggesting that there may be some clinical benefit worthy of larger clinical trials. Also, since combination antiretroviral therapies are limited for economic, social, political and sometimes religious reasons to the privileged persons, consideration of the potential of this supplement remains important for the developing countries, particularly those in the sub-Saharan Africa. Because of certain limitations (small sample size, short duration, late stage of the infection), further study is needed to confirm the influence of the supplement on the immune status and haematological parameters.

The effect of jet versus ultrasonic nebulized pulmonary surfactant on the respiratory mechanics of detergent-damaged isolated perfused rat lung.

J.A. Syce and S. Parkar

School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa (e-mail:jsyce@uwc.ac.za).

The aims of this study were to establish a lung model having reproducible lung injury and lung function features of ARDS and to use this model to compare the effectiveness of exogenous surfactant administered by jet or ultrasonic nebulizer and deep breathing. A model of an isolated rat lung that was ventilated under negative pressure and perfused with artificial medium while tidal volume (TV), lung compliance (CL), lung resistance (RL) and pulmonary artery pressure were continuously monitored was established. Aerosolized dioctyl sulfosuccinate (OT) was administered via the inhalation route to induce lung injury and then Survanta[R] aerosol prepared by jet or ultrasonic nebulizer was administered Two min inhalation of OT produced consistent decreases in TV (65% to 0,68 ±0.073 ml), CL (41% to 0.11±.004 cmH_20/ml), and RL (37% to 0.09±0.0169ml/cm H_20) with minimal change in pulmonary artery pressure (PAP). Administration of jet nebulized Survanta[R] did not improve the lung parameters even under deep breathing. Surfactant administered by ultrasonic nebulizer caused 130% (to 1,26±0.056ml) increase in TV, 82% (to 0.136±0.095ml/cmH20) increase in CL, but did not alter RL or PAP in the damaged lung. The findings suggest that administration of ultrasonic nebulized surfactant is better than jet nebulized pulmonary surfactant to reverse detergent induced lung damage resembling ARDS.

Changes in Prescribing Patterns of Selective Serotonin Reuptake Inhibitors Over a Six-Year Period

M. Bellingan, I.C. Wiseman, I.Truter and D.J.L. Venter#

Departments of Pharmacy and [#]Mathematical Statistics, University of Port Elizabeth, P.O. Box 1600, Port Elizabeth, 6000, South Africa. (e-mail:michelle.bellingan@upe.ac.za)

The aim of this study was to conduct a retrospective drug utilisation review to investigate changes in the prescribing patterns of selective serotonin reuptake inhibitors (SSRIs) in two elderly populations (1996; 2002) registered with a pharmaceutical benefit management company.

There were 1 172 and 2 640 depressed patients in 1996 and 2002, respectively. SSRIs accounted for 27.1% (205/ 756) of all the antidepressants prescribed in 1996. This representation increased to 48.4% (1 260/ 2 601) in 2002. This increasing therapeutic dominance of the SSRIs was associated with a significant reduction in the prescription of tricyclic antidepressants (1996: 50.7%; 2002: 34.1%) ($\lambda^2 = 69.31$, $p < .01$).

In 1996, paroxetine and fluoxetine were the most prescribed SSRIs accounting for 33.7% and 32.7%, respectively. Fluoxetine

should be used with caution in elderly patients due to its prolonged duration of action and high propensity for cytochrome P450 interactions. It was positive to note that citalopram, the SSRI regarded as the most appropriate in elderly patients, was the most prescribed in 2002 (35.6%). Fluoxetine, however, still accounted for 28.5% of all the SSRIs prescribed.

The adverse effect profiles and potential for drug-drug interactions of the respective SSRIs should be carefully considered to ensure selection of the most appropriate drug in the elderly depressed patient.

Prescribing patterns of methylphenidate in South Africa

I. Truter

Department of Pharmacy, University of Port Elizabeth, P.O. Box 1600, Port Elizabeth, 6000, South Africa

Methylphenidate is primarily used for narcolepsy and attention deficit hyperactivity disorder (ADHD) in children. The primary aim was to investigate the prescribing patterns of methylphenidate in a defined patient population in South Africa using pharmacoepidemiological methods.

A retrospective drug utilisation study was conducted. Data were obtained from a South African medical aid administrator. Prescription records for 115 patients who received methylphenidate during 2002 were analysed.

Nearly three-quarters (73.0%) of the patients were males and 79.1% of patients were 18 years or younger. The average age of patients was 15.6 (SD=10.8) years. Most patients were single and were child dependants. Seven patients were 40 years or older. Two-thirds of prescriptions (67.3%) were for methylphenidate 10 mg (the innovator product), 20.7% were for the 20 mg slow-release innovator product and the rest were prescriptions for the recently introduced 10 mg generic equivalent tablet. Patients received on average 4.0 (SD=3.3) prescriptions for methylphenidate during the year. Proportionately more prescriptions (44.4%) were dispensed during the period August to November. Just over 60% of all prescriptions were dispensed as 30 tablets (i.e. one tablet per day). The medicine categories frequently prescribed with methylphenidate were penicillins, antiepileptics and analgesics.

Further studies are recommended, especially into the quality of life of patients before and after receiving methylphenidate. The impact of methylphenidate on the academic performance of patients should also be quantified.

The Influence of Surfactants on Permeability of Chemical Markers Through Porcine Lung and Buccal Mucosa as well as Human Vaginal Mucosa.

J.M. Van Zyl, I. Viljoen, P. Van Der Bijl, A.D. Van Eyk and J. Smith

University of Stellenbosch, Tygerberg 7505, South Africa.

Lung surfactant is a complex mixture of phospholipids and four surfactant proteins (SP-A through SP-D) that provide it with unique spreading properties and dynamic surface tension behavior. Although the lipid and protein composition appears to be tissue dependent, the idea to use it as a carrier for therapeutic agents, provides an attractive way of drug delivery.

The objective of this study was to investigates the role of synthetic surfactants in the permeability of chemical markers through porcine lung / buccal mucosa and human vaginal mucosa.

Synthetic surfactant formulations were prepared by ultrasonic dispersion of phospholipids in saline. Using it as a vehicle, the flux rates of [^{3}H]-labeled forms of 17β-estradiol and arecoline through porcine lung, buccal mucosa as well as human vaginal mucosa were determined by using a continuous flow-through diffusion system. Mean steady state flux values were compared statistically using a t-test at a significance level of 5%.

In vitro, surfactants cause a statistically significant increase in permeation of 17β-estradiol / arecoline across porcine buccal mucosa, lung tissue as well as across human vaginal mucosa.

In conclusion, lung surfactant is an effective vehicle in the permeation of chemical markers through the tissues tested. The clinical relevance as a drug delivery agent warrants further investigation.

The prevalence of side effects: Ciprofloxacin 500 mg single dose prophylaxis against Neisseria Meningitidis outbreak in Potchefstroom during July 2003

M. Viljoen*, M.S. Lubbe, C.L Meyer***

Department of Pharmacology* and Pharmacy Practice**, Potchefstroon Campus (PUK), North-West University. Private Bag X2006, Potchefstroom, 2520 (fklmv@puknet.ac.za)

Potchefstroom experienced an outbreak of *Neisseria meningitidis* (*N. meningitidis*) during May-July 2003. An opportunity for obtaining valuable data arose when mass prophylactic treatment to approximately 28% of the Potchefstroom community was provided by the Department of Health, Northwest Province.

The aim of this study was to investigate the prevalence of side effects experienced by staff and students of the Potchefstroom University for Christian Higher Education who received a single prophylactic dose of oral ciprofloxacin 500 mg between 23 and 29 July, 2003. Information gained from the Potchefstroom outbreak may be valuable for the future management of similar outbreaks in other communities. Various stakeholders have published related reports, protocols, recommendations and guidelines, which mostly focused on the prevention, management and control of meningococcal disease. Very little has been reported about the side effects experienced, especially in cases where ciprofloxacin 500 mg single dose had been dispensed. One or more side effects were reported by 24.2% of the respondents, while 5.4% had to consult with a health care worker due to the severity of side effects resulting from a single dose. Practical significance could not be demonstrated for any of the side effects reported after single *vs.* multiple doses nor when the effects of gender or requirement for medical consultation were tested.

Validation of a modified method for the analysis of corticosterone in rat serum by high-performance liquid chromatography

F.P. Viljoen, L. Brand, B.H. Harvey

Department of Pharmacology, Potchefstroom Campus (PUK), North-West University,

Private Bag X2006, Potchefstroom, 2520 South Africa (e-mail:fklfpv@puk.ac.za).

Part of the physiological response to stress in rats is an increase in circulating levels of corticosterone. Determination of these levels in our laboratory was done up to now by means of radioimmunoassay techniques (RIA) and in order to make these measurements more cost-effective, the aim of this study was to develop our own sensitive and specific HPLC-method for corticosterone measurements.

The method of Wong et al 1994[1] was revised and modified for our conditions. Rat serum which was pooled and treated with decolorizing carbon to remove the endogenous corticosterone was used to prepare standards. A 500 µl sample was extracted with 5 ml dichloromethane and this extract was then analyzed by HPLC on a C_{18} column with a diode array detector at 245 nm. The detection limit of this method is 10 ng/ml. The standard curve was linear over the concentration range of 10 – 500 ng/ml (regression 0.9998). Corticosterone recovery after extraction ranged from 86 to 100%. The relative standard deviation for repeatability of the concentrations 10, 100 and 500 ng/ml was 8.8, 3.0 and 6.0 respectively. Results from this method was then compared with values obtained from a RIA method. Corticosterone levels of ten rats were also measured by using both methods and the values were in close proximity of each other.

We therefore conclude that the HPLC method as modified in our laboratory is both sensitive and specific for the measurement of

corticosterone levels.

1. Wong Y.N. et al (1994). *J. Chromatogr. B*, 661, 211-218.

The Effect of Liposomal Surface Charge on the Disposition of Liposome-Encapsulated Gentamicin to the Rat Liver, Brain, Lungs and Kidneys after Intraperitoneal Administration.

AM Abraham and A Walubo

Department of Pharmacology, University of the Free State, South Africa.

Introduction and aim: Although gentamicin is one of the most well studied drugs in liposomal formulations, delivery of gentamicin to diseased tissues remains a daunting task owing to its highly charged large molecule that retards its movement across cell membranes. It is still not clear which liposomes, with regard to surface charge, would best serve for severe infections of major organs such as the liver, kidney, lungs and brain. As such, this study was undertaken to evaluate the effect of liposomal surface charge on the distribution of liposome encapsulated-gentamicin to the brain, kidney, liver and lungs of a rat.

Methods: Four groups of 25 rats each were administered intraperitoneally with 60 mg/kg of either free gentamicin (control group) or gentamicin encapsulated in negative, positive and neutral liposomes. Thereafter, for each group, five animals were sacrificed at intervals of 1, 2, 4, 6 and 8 hours and the four organs were removed and analysed for gentamicin. The disposition of gentamicin to the normal rat brain, lung, kidney and liver was studied over the eight hours period.

Results: In comparison to the control group, liposomes were associated with higher concentrations of gentamicin in the brain and liver, while concentrations were lower in the kidney. The average concentrations of gentamicin in the liver and the brain were highest with positive liposomes, while, gentamicin concentrations in the kidneys and lungs were not influenced by surface charge of the liposomes.

Conclusion The surface charge of liposomes is an important determinant of the disposition of liposome-encapsulated gentamicin to the brain and the liver, and the intraperitoneal route can be reliably used for study of kinetics of liposome-encapsulated gentamicin.

The Role of CYP3A in Nevirapine Induced Hepatotoxicity

S Barr, and A Walubo

Department of Pharmacology, University of the Free State

Introduction and aim: Nevirapine is a potent non-nucleoside reverse transcriptase inhibitor with favourable pharmacokinetics that are characterised by rapid absorption and distribution with a long elimination half-life. Nevirapine is effective against HIV-1 when used in combination with other anti-retroviral agents, and as a monotherapy for the prophylaxis of mother-to-child HIV-1 transmission. Unfortunately, the wide use of nevirapine is hampered by its adverse effects, mainly hypersensitivity skin reactions and hepatotoxicity. Since nevirapine induced hepatotoxicity commonly occurs between 2 – 12 weeks of treatment, and nevirapine is a known inducer of CYP3A iso-enzyme, it was envisaged that the hepatotoxicity is due to activation of nevirapine to toxic metabolites by the induced enzymes. Therefore, the aim of this study was to determine the role of CYP3A in nevirapine induced hepatotoxicity.

Method: Three groups of five SD rats each were pre-treated with nevirapine (20 mg/kg) orally for three days to induce CYP3A. On the fourth day, group A (control) was administered the vehicle, while Group B was administered a toxic dose of nevirapine (1340 mg/kg), and Group B was administered ketoconazole (20 mg/kg) intraperitoneally, a CYP3A inhibitor, followed by the toxic dose of nevirapine one hour later. The rats were sacrificed 24 hours after treatment, blood was sent for liver function tests and the livers were saved for microsomal extractions and histology. Microsomal CYP3A activity was measured by the erythromycin demethylation test while quantitation was by SDS-PAGE and western blot. Results of these animals were compared with results of animals that were not pre-treated with nevirapine.

Results: Pre-treatment with nevirapine lead to induction of CYP3A. CYP3A activity in the untreated group was 0.59 ± 0.48 nmol/min/mg versus 7.28 ± 2.65 nmol/min/mg after treatment. There was heptotoxicity in group B, but hepatotoxicity was not prevented by ketoconazole in group C. Liver function tests were: ALP 167.6 ± 37.105 U/L, AST 150.2 ± 19.11 U/L and ALT 67.2 ± 8.64 U/L for group B, and ALP 211.4 ± 30.85 U/L, AST 258.8 ± 192.86 U/L and ALT 198.2 ± 154.36 U/L for group C. Interestingly, there was no hepatotoxicity when the toxic dose of nevirapine was administered to animals that were not pre-treated with nevirapine.

Conclusion: Nevirapine induced hepatotoxicity is associated with enzyme induction but CYP3A is not involved its pathogenesis, and this suggests that a different enzyme may be responsible.