The Journal of Applied Therapeutic Research focuses on the efficacy, safety, and rational use of drugs once they have been approved by regulatory authorities for general marketing.

The journal publishes original research papers and short communications which have been refereed, plus commissioned review articles. Coverage includes: drug efficacy, drug safety and risk assessment, therapeutic drug monitoring and clinical pharmacokinetics, patient compliance, drug use evaluation, drug information services, drug misuse and abuse, health related quality of life and pharmacoconomics.

Guide for Authors can be found at the back of the journal.
A CD-ROM ARCHIVE version in a pdf format is available for this journal at a nominal extra charge to subscribers.

Abstracts Services
Journal of Applied Therapeutics is abstracted in International Pharmaceutical Index (www.ashp.org).

World Wide Web Addresses
Additional information is also available through the Publisher’s web home page site at http://www.euromed.uk.com.
Editorial enquiries by e-mail: info@euromed.uk.com

Ordering Information
Four issues per volume. Subscriptions are renewed on an annual basis.

Orders may be placed with your usual supplier or at one of the addresses shown below. Journal subscriptions are sold on a per volume basis only; single issues of the current volume are not available separately. Claims for non receipt of issues will be honoured if made within three months publication of the issue. See Publication Schedule Information.

All issues are dispatched by airmail throughout the world.

Subscription Rates
Personal Subscription price per volume; £75.00. This price is available only to individuals whose library subscribes to the journal OR who warrant that the journal is for their own use and provide a home address for mailing. Orders must be sent directly to the Publisher and payment must be made by personal cheque or credit card.
Institutional Subscription price per volume; £150.00. Subscribers should contact their agents or the Publisher.

Ordering Information
Orders should be placed through one of the addresses below:
Euromed Communications Ltd
The Old Surgery, Liphook Road, Haslemere,
Surrey GU27 1NL, UK
Tel: +44-(0) 1428 656665   Fax: +44-(0) 1428 656643

Nankodo Distribution Agency, Japan
Voice: +81 3 3811 9950
Fax: +81 3 3811 5031
E-mail: nkdyosho@nankodo.co.jp
Website:www.nankodo.co.jp

Orders or enquiries can also be sent by e-mail: info@euromed.uk.com and the world wide web:
http://www.euromed.uk.com

©2005 Euromed Communications Ltd. No part of this publication may be reproduced or transmitted in any form or by any means, electronic, mechanical, photocopying or otherwise, stored in a retrieval system of any nature, without the advance written permission of the Publisher. However, institutions with a photocopy licence may make copies as prescribed by the Copyright Clearance Centre in the USA or the Copyright Licensing Agency in the UK.

ERRATUM


‘…The indices of fractional reduction of the area under the parasite density time curve at 24h and 48h were less than 0.6 and 0.8, respectively. Delayed parasite clearance after 3 or 4 days of chloroquine-treated P. falciparum in children aged less than 3 years with a presenting temperature >39°C and increased parasitaemia at 24 hours may be predictive of chloroquine resistance. These indices may be useful when evaluating and monitoring resistance in children before clinical manifestation.'
Clinical benefits of patient medication notebooks

SHUN-ICHI TANAKA¹, AKIO KAWACHI², RYOKO KAI¹, TOMOHISA HASEGAWA¹, KYOKO HISAMI¹, YUKO YONEDA¹, AKITO SUZUKI³, HIDEMITSU INADA¹, NORIYUKI KURASAWA¹, MASAMI HIRAI³, TOSHIRO MOTOYA²*

¹ The Pharmaceutical Association of Nobeoka City, 3-5-9, Ohse-machi, Nobeoka, Miyazaki 882-0841, Japan.
² First Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1, Yoshino-machi, Nobeoka, Miyazaki 882-8508, Japan.
³ Department of Clinical Pharmaceutics, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1, Yoshino-machi, Nobeoka, Miyazaki 882-8508, Japan.

*Correspondence: Toshiro Motoya, First Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1, Yoshino-machi, Nobeoka, Miyazaki 882-8508, Japan.
Tel: +81-982-23-5529; Fax: +81-982-23-5536; e-mail: motoya@phoenix.ac.jp

Pharmacy practice includes finding and solving drug therapy problems, and making patients aware of the risks of taking prescribed medicines. Provision of written drug information such as patient information leaflets (PILs) has become popular nationwide. In addition, a medication history record system for patients called 'the patient medication notebook' (PMN) has also been devised and utilized in Japan. We have recently conducted a questionnaire survey to discover the clinical benefits of patients using a PMN. We analyzed data collected from 51 community pharmacies. From a total of 12,470 patients recruited in these pharmacies some 37% (4,599/12,470) were found to use a PMN. Most importantly, examination of these PMNs enabled 70 pharmaceutically questionable incidences to be identified in the 4,599 PMNs. These included 34 combined drug treatments where a drug was contraindicated or where a medicine needed to be used with caution, and 31 duplicate treatments where the same medicines or the same category of medicines was being prescribed. Finally, in 33 cases, a prescription was changed after intervention by the pharmacist following a problem which was detected in the PMN. From our results, we have been able to confirm the clinical benefits of patients using a PMN. The next phase is to promote the use of the PMN more widely.

Keywords: patient medication notebook, medication history, drug interaction, duplicate medication
**INTRODUCTION**

Pharmacy practice includes finding and solving drug therapy problems, and making patients aware of the risks of taking prescribed medicines (McDonough, 1999; Hermansen-Kobulnicky et al., 2004). Importantly, patient information leaflets (PILs) provided in community pharmacies help to bridge the information gap between the physician and patient (Gibbs et al., 1990; Mottram et al., 1997), and studies have shown that PILs improve a patient’s understanding of medicines and administration procedures (Buck, 1998; Sata et al., 2003; Svarstad et al., 2004). The percentage of patients receiving computer-generated PILs provided in pharmacies has been increasing in Japan, since information about prescribed medicines has to be supplied by pharmacists to comply with the law in Japan (1996).

Elderly patients are often faced with polypharmacy when they experience multiple disease processes (Patel, 2003). The size of the elderly population (aged 65 or over) was 20% of the total Japanese population in 2004, although it was only 12% in 1990, according to data presented by the Statistics Bureau, Ministry of Internal Affairs and Communications of Japan on 15th September 2004. Elderly patients may often receive treatment in two or more specialist hospitals for their multiple diseases (Nakamura et al., 1990), and unfortunately the medical staff may fail to detect duplicate medication or combined treatments of medicines which may cause drug interactions etc. Therefore, the medication history record system called ‘the patient medication notebook’ (PMN) as shown in Figure 1 has been devised and utilized in Japan (Ishibashi et al., 1990; Iga, 1999), and the contents of prescriptions are recorded at every visit to the community pharmacy or the hospital pharmacy. Thus the PMN acts as a checkable medical record of drugs prescribed by multiple facilities.

Adequate patient information is important for high quality care, and it is one of the key indicators of patient centeredness, improving the effectiveness and efficiency of care (Buck, 1998; Stoop et al., 2004). Coupled with the population’s increasing awareness of medical care, provision of written drug information such as PILs, has become highly popular nationwide (Motoya et al., 2003; Sata et al., 2003; Svarstad et al., 2004). Also in Japan, it appears that most patients want detailed information about their medication, that they favour the provision of PILs, and that they are satisfied when understandable PILs are provided. However, little is known about the quality and potential benefits of the PMN being disseminated in Japan, and we have found no published studies that are sufficiently specific, comprehensive, and scientifically accurate.

Thus, the objective of this study was to investigate the existing dissemination of the PMN, and evaluate whether it is beneficial for the patient.

**METHODS**

The study was conducted between 12th and 17th July 2004, and consisted of a questionnaire survey to evaluate the clinical benefits of patients using a PMN at community pharmacies in Nobeoka city, Miyazaki prefecture, Japan. An overview of the questionnaire used in this study can be seen in Table 1. The questionnaire was constructed to answer 3 main questions:

1) What percentage of patients take their PMN to the community pharmacy?
2) How many instances of pharmaceutically questionable decisions can be detected in the PMN?
3) What is the benefit to the patient of using the PMN? The questionnaires were sent by mail to 61 community pharmacies, belonging to the Pharmaceutical Association of Nobeoka City.

RESULTS

Of the questionnaires distributed to 61 community pharmacies, 51 (83.6 %) produced usable responses, reporting on a total of 12,470 patients registered in these pharmacies.

PMN USERS

The proportion of patients who used a PMN was 37% of all pharmacy visitors (Figure 2). Of the 63% non-users, 19% were in possession of a PMN, but did not use it. In the PMNs of 4,599 users, we found that 29% of patients were being treated concurrently at two or more hospitals in the same month. Overall, 94% of PMN users were found to utilize the PMN properly, although 6% of patients were using two or more PMNs from different hospitals at the same time.

Use of the PMNs by pharmacists

Of the 4,599 PMNs studied, 1.5% (70 cases) were found to contain a pharmaceutical administration problem (Table 2). Of these 70 'questionable' cases, 34 consisted of combined treatments of medicines which were contraindicated or which should be used with caution. Table 3-A shows examples of these cases. There were also 31 cases of duplicate treatments (Table 2). As shown in Table 3-B, these were categorized as follows: a-e) the same medicine being prescribed to the patient in duplicate, f-h) medicines of the same category being prescribed in excess. Five cases revealed other problems. One case in which the adverse effect of a medicine prescribed separately was discovered on

Table 1. The items of the questionnaire to evaluate the PMN

<table>
<thead>
<tr>
<th>The PMN users</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total no. of patients who visited the community pharmacy</td>
<td></td>
</tr>
<tr>
<td>2. No. of PMN users</td>
<td></td>
</tr>
<tr>
<td>3. No. of hospitals that the PMN users consulted</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The detection of pharmaceutically questionable cases in the PMN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total no. of questionable cases detected in the PMN</td>
<td></td>
</tr>
<tr>
<td>2. Example of questionable cases in the PMN</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The clinical benefits of the PMN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No. of questionable cases that were resolved by the pharmacist’s knowledge</td>
<td></td>
</tr>
<tr>
<td>2. No. of cases that required the pharmacist to consult the physicians</td>
<td></td>
</tr>
<tr>
<td>3. No. of cases in which the prescription was changed after inquiry by the pharmacist</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The number of pharmaceutically questionable cases detected in the PMNs

<table>
<thead>
<tr>
<th></th>
<th>Percentage (No. of detected/ no. of PMN user)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total detected pharmaceutically questionable cases</td>
<td>1.5% (70 / 4,599)</td>
</tr>
<tr>
<td>Combined treatments of medicines with contraindications or that should be used with caution</td>
<td>0.7% (34 / 4,599)</td>
</tr>
<tr>
<td>Duplicate treatments with the same medicines or the same category medicines</td>
<td>0.7% (31 / 4,599)</td>
</tr>
<tr>
<td>Others</td>
<td>0.1% (5 / 4,599)</td>
</tr>
</tbody>
</table>

Figure 2. The proportion of PMN users and non-users in Nobeoka city (12th-17th July 2004).
Table 3. Examples of pharmaceutically questionable cases detected in the PMN

A. Combined treatments with medicines with contraindications or that should be used with caution

<table>
<thead>
<tr>
<th>Detected pharmaceutically questionable cases</th>
<th>Details of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescribed medicine</td>
<td>Co-administered medicine</td>
</tr>
<tr>
<td>a. Anti-diabetic medicines (not specified)</td>
<td>Gatiflo® (gatifloxacin)</td>
</tr>
<tr>
<td>b. Xalatan® Eye Drops (latanoprost)</td>
<td>Buscopan® (scopolamine butylbromide)</td>
</tr>
<tr>
<td>c. Ubretid® (distigmine bromide) and Harnal® (tamsulosin hydrochloride)</td>
<td>Ludomiil® (maprotiline hydrochloride)</td>
</tr>
<tr>
<td>d. Warfarin (warfarin potassium)</td>
<td>NSAID (not specified)</td>
</tr>
<tr>
<td>e. Avishot®Å@ (naftopidil) and Harnal® (tamsulosin hydrochloride)</td>
<td>anti-hypertensive medicine (not specified)</td>
</tr>
<tr>
<td>f. Itrizole® (itraconazole)</td>
<td>Medicines for stomach (not specified)</td>
</tr>
<tr>
<td>g. Anti-diabetic medicine (not specified)</td>
<td>NSAID (not specified)</td>
</tr>
</tbody>
</table>

B. Duplicate treatments

<table>
<thead>
<tr>
<th>Detected pharmaceutically questionable cases</th>
<th>Details of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescribed medicine</td>
<td>Co-administered medicine</td>
</tr>
<tr>
<td>a. Methycobal® (mecobalamin)</td>
<td>Methycobal® (mecobalamin)</td>
</tr>
<tr>
<td>b. Methycobal® (mecobalamin)</td>
<td>Methycool® (mecobalamin)</td>
</tr>
<tr>
<td>c. Selbex® (teprenone)</td>
<td>Selbex® (teprenone)</td>
</tr>
<tr>
<td>d. Zaditen® Syrup (ketotifen fumarate)</td>
<td>Zaditen® Syrup (ketotifen fumarate)</td>
</tr>
<tr>
<td>e. Loxonin® (loxoprofen)</td>
<td>Loxonin® (loxoprofen)</td>
</tr>
<tr>
<td>f. Loxonin® (loxoprofen) (surgery and urology)</td>
<td>Voltaren® (diclofenac Na)</td>
</tr>
<tr>
<td>g. H2 blocker (not specified)</td>
<td>H2 blocker (not specified)</td>
</tr>
<tr>
<td>h. Antibiotic (not specified)</td>
<td>Antibiotic (not specified)</td>
</tr>
</tbody>
</table>

C. Other

<table>
<thead>
<tr>
<th>Detected pharmaceutically questionable cases</th>
<th>Details of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescribed medicine</td>
<td>Co-administered medicine</td>
</tr>
<tr>
<td>Shosaikoto extract granule (Chinese traditional herbal medicine)</td>
<td>Proteolytic enzyme</td>
</tr>
</tbody>
</table>
examination of the PMN is shown in Table 3-C. Of the 70 problems detected, 30 cases were resolved by the pharmacist based on their medication knowledge. For the other 40 cases, it was necessary to consult with a physician (0.9%). Finally, in 33 cases, the prescriptions were changed after intervention by the pharmacist. The results of the survey are summarized in Figure 3.

**DISCUSSION**

A study of patient PMNs revealed 70 potential problems of a pharmaceutical nature in the 4,599 PMNs examined over a study period of 6 days (1.5% of all PMN users). The 70 cases included 34 drug combinations where one or more drugs were contraindicated or should be used with caution, and 31 examples of duplicate treatments with the same medicines or the same category of medicines. Examples of the former group included:

i) gatifloxacin which is contraindicated in diabetes mellitus being prescribed to a diabetic patient,
ii) scopolamine butylbromide that may cause an increase in intraocular pressure prescribed to a patient with glaucoma or ocular hypertension using latanoprost eye drops,
iii) maprotiline hydrochloride which may cause dysuria prescribed to a benign prostatic hyperplasia patient being treated with distigmine bromide and naftopidil,
iv) an anti-hypertensive medicine prescribed to a patient being treated with naftopidil and tamsulosin hydrochloride which may lead to hypotension.

Furthermore, duplicate treatments included the excessive administration of medicines such as mecobalamin, teprenone, ketotifen fumarate, an analgesic, H$_2$ blocker and an antibiotic. In another case, a proteolytic enzyme was prescribed to a patient with swelling when Shosaikoto extract granule which can cause swelling had been prescribed by another hospital. et al. In this study, use of PMNs succeeded in improving the following patient’s medication:

i) in 33 cases (0.7%) the prescription was changed after intervention by the pharmacist,
ii) in 30 cases (0.7%) a questionable prescription was detected and resolved based on the pharmacist’s knowledge.
In these cases, the patients had received treatment at two or more hospitals where the medicines had been prescribed separately. The questionable cases mentioned above would be difficult to detect without the use of a PMN, confirming that PMNs are clinically beneficial to the patient.

The cases detected may represent only 'the tip of the iceberg', because only 37% of all patients were found to use their PMNs in this study, and it is easy to imagine that the actual number of interventions would be considerably increased if all patients used a PMN. Clearly the use of PMNs will help to prevent side effects and the duplication of prescribed medicines and, thus will be of benefit to patients. Therefore, PMN use needs to be promoted more vigorously. One possible reason for the low percentage of patients using a PMN at present is thought to be a lack of patient awareness about the risk of taking medicines prescribed by multiple facilities. Hence, patient education about the benefits of PMNs may help to solve the problem. The next phase is to inform patients of the benefits of using a PMN and to promote its use.

In conclusion, examination of PMNs resulted in 70 pharmaceutically questionable cases being identified in the PMNs of 4,599 patients. In 33 cases, the prescriptions were changed following the intervention of a pharmacist. These results confirm the benefits of patients using PMNs.

**Acknowledgements**

This study was conducted in cooperation with the members of the Pharmaceutical Association of Nobeoka city.

**REFERENCES**


There are approximately 45 million uninsured individuals in the USA. Although many barriers to health care have been identified, one significant barrier is access to prescription drugs. Most uninsured individuals are unable to pay for their prescriptions. In many cases, patients postpone, prioritize, ration, and substitute prescriptions. We conducted a survey to obtain information from several programs in the United States to determine their strategies for offering prescription drugs to the uninsured or indigent populations. Manufacturers’ medication assistance programs (MAPs) were frequently used by these agencies. Additional strategies included reduced copay, samples, vouchers, rebates, and medications obtained by the clinics through federal qualified programs. A variety of educational programs were offered by these centers. Despite these efforts, there is a great need for additional and continued funding of programs to offer an adequate supply of prescription drugs to this population and to assure that the desired outcomes of therapy are achieved.

Keywords: Indigent, Uninsured, Medication Assistance Programs, Prescription Access
BACKGROUND

Limited access and affordability of health care are of major concern to the public. The current health care system does not appear to meet the needs of many individuals, especially the indigent and uninsured patient populations. This is even a greater concern with the aging population. In 2003, the number of people without health insurance in the United States was 45 million; the percentage of the total population without coverage rose from 15.2% in 2002 to 15.6% in 2003 according to the United States Census Bureau News published in 2004. Approximately 40 million Americans are covered by the nation’s largest health insurance program, Medicare, which provides health insurance to people of age $\geq 65$ years, to those with permanent kidney failure, and to certain people with disabilities. Most prescription drugs are not covered under Medicare leaving this population without prescription drug coverage (Medicare and Medicaid Services, 2003). Considering all categories, many Americans do not have prescription drug coverage. Current barriers for prescription access

Among the many barriers that indigent patients face in accessing prescriptions, the most obvious one is of financial origin. People just cannot afford their medications. Both rural and urban communities face underemployment and poverty leading to the inability to obtain prescribed pharmacotherapy. The urban communities seem to have a higher percentage of individuals covered by entitlement programs or with other resources than their rural counterparts (Strickland & Hanson, 1996).

Patients have many coping strategies to deal with the financial barriers for obtaining prescription drugs. Financing by some mechanism and postponing purchase of prescriptions were the top strategies used by this population to cope with the cost of prescription drugs. There were several financing strategies including charging prescriptions at the pharmacy by a credit card or an account, borrowing money, having someone pay for their medications, and seeking professional samples or free medication. Many patients would postpone filling their prescription until they have received a monthly paycheck or have saved enough money. Some individuals would attempt to obtain a nonprescription substitute or defer going to a physician until they could afford a prescription. Other coping strategies included prioritizing, rationing, and substituting prescriptions (Strickland & Hanson, 1996). Patients would set priorities to determine what was more important: filling their prescription or buying groceries for sustenance. Some would rationalize whether the drug was even worth obtaining to treat their illness or disease. Lastly, patients would just substitute their prescription with a less costly means. For example, a patient may purchase nonprescription acetaminophen for the prescription drug, acetaminophen with codeine.

In July 2000, the Prescription for Care (PFC) Alliance was formed in Franklin County, Ohio. The alliance consists of 34 agencies including local pharmacies, community outreach programs, local hospitals and health centers (Table 1). Among these agencies there are eight lead organizations, one of which is the Ohio State University College of Pharmacy (Table 2). The governing organization for PFC is the Columbus Neighborhood Health Center, Inc. The goal of PFC is to improve prescription access to the uninsured and lower-income individuals without prescription drug benefits. According to a 1997 report, there were approximately 117,000 uninsured individuals (11.6% of the population) in Franklin County of Ohio. Among these individuals, eight percent were children. Furthermore, most were young, Caucasian, working adults with low incomes, as seen at the national level (Lewin Group, 1997). PFC is funded through a grant from the Columbus Medical Association Foundation. In order to develop an improved prescription access program, PFC conducted a survey of other assistance programs for prescription medications around the country.

MODELS BEING UTILIZED ACROSS THE NATION

During the development phase of PFC, several programs were identified as potential best practices across the United States. The goal was to model our program with the best features of existing programs. In particular, programs were targeted to obtain...
specific information about prescription access. These consisted of Columbus Neighborhood Health Center, Inc., Columbus, Ohio; Indian Health Services; Grady Health System, Atlanta, Georgia; CareLink Program, San Antonio, Texas; Colorado Indigent Care Program, Denver, Colorado; Elderly Pharmaceutical Insurance Coverage Program of New York; Buncombe County, North Carolina; Capital Area Prescription Program, Ingham County, Michigan; MedBank of Maryland, Baltimore, Maryland; and Durham County, North Carolina.

**COLUMBUS NEIGHBORHOOD HEALTH CENTER, INC.**

The Columbus Neighborhood Health Center, Inc. (CNHC) in Columbus, Ohio offers health care at eight clinics to uninsured and indigent populations in Franklin County and surrounding communities. Patients are entered into the system on a sliding scale fee schedule if they do not qualify for assistance through state and federal programs. CNHC has eight clinics, with physicians, nurse practitioners, registered nurses, medical assistants, and clinical pharmacists on staff. Everyday, practitioners face challenges to obtain prescription medications for their patients.

The clinics are accessible to all who need health care. The majority of services offered are in adult primary care but three clinics have also implemented pediatric, and obstetrics/gynecology services. In addition, one clinic has dentistry and optometry services. Patients are seen by providers and are charged a fee. The fee schedule determines the payment required by the individual to obtain prescriptions (Table 3). If prescriptions are needed, several options are used for patients to receive their medications. Primarily, CNHC uses a negative formulary system. This is a list of medications that cannot be prescribed to their patients. If a drug is needed for the patient but not on this formulary, then
a prior authorization must be obtained from clinical pharmacist to receive the agent. CNHC contracts with a pharmacy benefits manager (PBM), USI Rx, which informs the pharmacy about the cost to the patient based on their fee schedule. CNHC also uses other methods to obtain medications for patients that include pharmaceutical manufacturer medication assistance programs (MAPs), Pfizer’s Sharing-the-Care® program, physician bulk dispensing, clinic stock, and pharmaceutical manufacturer samples.

INDIAN HEALTH SERVICES

The Indian Health Service (IHS) is a federal agency within the Department of Health and Human Services. IHS provides health services to American Indians and Alaska natives. Currently, over 1.5 million individuals benefit from their services and more than 550 federally recognized tribes in 35 states use this service. The main limitations this population faces in accessing prescription medications are lack of transportation and inadequate funding but the public health nurses and local health service representatives are addressing these. Pharmacists offer educational programs and provide pharmaceutical care to patients.

GRADY HEALTH SYSTEM

Grady System primarily deals with the indigent populations in Fulton and Dekalb counties of Georgia. Currently, it provides healthcare to approximately 40,000 institutionalized inpatients and over 750,000 ambulatory patients per year. There is an estimated 150,000 individuals that need assistance with prescription drugs. One of the main barriers that patients face when attempting to access prescriptions in this system is long wait times in clinics and at pharmacies. This is partially due to inadequate space or staff to serve this population. Grady is in the process of contracting for automation services in pharmacy and improving their telephone refill system to decrease the wait time for patients.

The taxpayers of Dekalb and Fulton County Georgia subsidize prescription funding for patients. Patients at or below 125% of poverty level pay only fifty cents per prescription. In addition, approximately eight major pharmaceutical manufacturers provide some support to the system. In 2000, Grady received support valued at $2,000,000. In a typical month, pharmaceutical manufacturer MAPs provide over 9,000 prescriptions. Clinical pharmacists offer educational programs to patients. In particular, the areas of diabetes mellitus, proper inhaler use, hypertension, hyperlipidemia, and lifestyle changes are addressed. Barriers to prescription access may decrease due to increased number of staff, improvements of facilities, and education of the community.

CARELINK

The CareLink® program is associated with University Hospital and University Clinics at The University of Texas Health Science Center in San Antonio, Texas. It currently serves 68,000 individuals in need for prescription access. Re-enrollment is done on an annual basis to verify the existing needs of the individual. The main barriers that patients confront when accessing prescription medications are timely access to reimbursement specialists and the difficulty in being enrolled into the program. Prescription access problems are addressed by use of manufacturer indigent MAPs, prescription co-payments, and various fund-raising activities. Some funds are available from hospital district taxes. Recently, staff has been added to facilitate the work required to obtain medications through MAPs. Education to patients is critical, especially to the high percentage of diabetic patients who live in South Texas. The physician, while seeing the patient, provides education on several issues. The success of this program to overcome barriers to prescription access is due to MAPs being used, co-payments for prescriptions, and extensive fund-raising. In addition,
educating providers about the existence of the program has saved over $2 million in 1999.

**ELDERLY PHARMACEUTICAL INSURANCE COVERAGE (EPIC)**

EPIC serves 154,000 indigent elderly people in the State of New York. There are over 200,000 individuals that need to be served by this program. State Legislature and rebates from drug manufacturers fund the program. Income limits have been expanded so many people are eligible for the program and are able to receive prescription medications. On average, EPIC pays 70% of the cost of a prescription and some patients pay a deductible, based on their income. Barriers that patients confront to obtain medications through this program are related to limited staffing and financial resources. Physicians and pharmacists offer educational programs to patients. The program’s success is linked to not having a restrictive pharmaceutical formulary and being senior friendly.

The Colorado General Assembly enacted the “Reform Act for the Provision of Health Care for the Medically Indigent” in 1983. The state’s non-Medicaid medically indigent residents benefit from this Act. Providers are reimbursed by state funds for services provided to patients. The CICP network is composed of 49 hospitals, 18 clinics, and 51 satellite facilities. University Hospital in Denver is one of these sites, which also operates a CICP pharmacy. The pharmacy dispenses medications obtained from pharmaceutical manufacturer MAPs. CICP is not a health insurance program nor a prescription program, but a means for providers to recover medical costs.

Currently, the CICP at University Hospital provides services to 6,100 individuals. Individuals requesting assistance are not always unemployed but may have insurance without adequate benefits. Many patients will delay seeking treatment for their illnesses for not being able to take time off from work or not having any remaining sick days. This leads to worsening signs and symptoms of their illness and complications. One major barrier that patients face to obtain medications through this system is the number of limited pharmacies at which they can have their prescription filled. In addition, there are no funds designated for prescriptions and most costs are absorbed by the hospital. Patients enrolled into the CICP system are placed on a fee for service basis, with co-payments for prescriptions ranging from $5 to $25. Approximately, $2 million worth of medicine is obtained through MAPs. The efficiency in receiving medications through MAPs is attained by the use of full-time pharmacy technicians, whose primary responsibility is to initiate and complete the paperwork for manufacturers. Pharmacists offer educational programs to patients and focus on diabetes and smoking cessation.

**BUNCOMBE COUNTY, NORTH CAROLINA**

The Buncombe County Health Center in North Carolina is one entity of the Buncombe County Medical Society Project Access. This is a partnership between the center, area hospitals and pharmacies, county government, and its Medical Society. This county has one of four health departments in the state to offer a full service primary care clinic to their community. Approximately, 70% of the patients in the program qualify for free medication on a sliding scale basis for being at or above 200% of the poverty level. In addition, there is an extensive effort to obtain medications through the MAPs.

Currently, 30,000 individuals are being served by the center. Funds for providing prescription access come from the Buncombe County Commissioner’s budget and limited grant money. The main barriers that patients face in accessing prescription medications are financial, transportation, lack of education, and language. These are being addressed by methods used in other programs, such as MAPs, sliding scale eligibility for free or reduced cost medications, and the use of Spanish and Russian translators or interpreters. Educational programs are offered to patients, which primarily consist of prenatal care and smoking cessation. A good community network and extensive education to providers about available resources have assisted Buncombe County to overcome common barriers to prescription access.
OTHERS:
Many of the other programs focused on overall healthcare rather than prescription care plans. However, some successful prescription models have been developed in Durham County North Carolina, Ingham County Michigan, and Medbank of Baltimore, Maryland. Medbank uses only MAPs to address problems with prescription access, while Ingham County uses PBM discounted pricing to improve prescription access. The Durham county model, like EPIC, has prescription assistance programs for seniors only, whereas Ingham and Medbank programs are designed for the general population.

LIMITATIONS OF THE CURRENT MODELS

MAPs
There are several ways to obtain information about MAPs. The most common method is directly from the drug manufacturer. The information is readily available by calling the company, using the company’s web site, or asking a sales representative. Most pharmaceutical manufacturers belong to the organization, Pharmaceutical Research and Manufacturers Association (PhRMA). This organization has published the Directory of Prescription Drug Patient Assistance Programs (2001-2002), which is a resource listing prescription assistance programs provided by manufacturers. The directory can be accessed on the Internet at http://www.phrma.org/searchcures/dpdpap. In addition, many other websites have been developed with the sole purpose of providing information about assistance programs. The two most common sites are www.needymeds.com and www.rxassist.org. Both provide information on how to obtain medication through MAPs. Some of the MAP forms are also provided on these sites. In addition to these sites, many organizations publish an annual listing of companies offering MAPs. A recently published journal article provides a list of common MAPs for uninsured and indigent patients (Chisholm et al., 2000).

One limitation to using the resources described above is that the information provided is not updated in a timely manner. Many MAPs expire as patents for products expire and generic drugs become available. As new products appear on the market, MAPs for these become available but often not immediately and with different criteria.

The majority of programs searched use manufacturer MAPs to obtain prescription medications for their patients. Typically, the product arrives to the physician’s office or the patient’s home and will last for up to ninety days. During this ninety-day period, a new form usually needs to be completed and mailed to obtain the next ninety-day supply. Often, patients forget to notify their doctor’s office well in advance before their medication needs refilled, which requires other resources for their medication during the interim period. In such cases, either the patient or the center has to make a financial commitment.

Another limitation to using MAPs is the amount of information that needs to be provided on the application. Obtaining this information can be tedious for the individual completing the form. Most of the program’s applications require both signatures from the patient and the provider, and patients must provide detailed financial information to prove their hardship. Some of the programs allow faxing the application with a turnaround time of two weeks, while others require them to be mailed which can take 6-8 weeks for the medication to be delivered to the patient or the physician.

The forms for MAPs are not standardized among manufacturers. Some applications consist of multiple pages versus those that are on a single page. Several companies only allow the use of an original application that were mailed to the physician's office and do not accept a photocopy. This causes a delay in processing these applications if the original forms are not available. In addition, many programs require that a physician or a physician’s agent call and provide their DEA number for the new application forms to be mailed to the office. Finally, there is often a limit to the number of applications, which can be ordered at one time.

Other Limitations
In the CNHC system, the Pfizer® Sharing-the-Care program offers certain products and it only allows
individuals, who are in sliding scale schedule B, to obtain medications. This program is available only to those health centers that qualify for the program. The physician bulk program often does not have enough medications to meet the demands of each clinic. The medications are ordered at a central office and then distributed among the clinics. The quantity ordered is predicted based on the previous quarter usage and does not account for new patients entering the system.

Sample medications are another means for the programs to provide medications to the uninsured or indigent patients. The sample supply is insufficient to meet the demands of the health centers. Often, there is inadequate supply to cover the patient until medication can be obtained through a MAP. Many pharmaceutical manufacturers are using the voucher system to help ease this problem. However, the center must often wait for a sales representative to bring vouchers to the clinic, which delays the time for a needed prescription to be filled.

The barriers seem to be similar for various programs. There is lack of money, transportation, insurance, and education among the patients. From the pharmacy perspective, limited number of participating pharmacies, inadequate amount of drugs, and insufficient staffing lead to prescription access problems. Finally, the outcomes of drug therapy among patients are not being evaluated.

CONCLUSIONS

Millions of Americans are unable to obtain prescription medications due to financial constraints. MAPs supported by the pharmaceutical companies are frequently offered to these patients. The educational programs offered by model agencies range from disease-specific programs, such as diabetes, to a focus on educating patients and providers with drug-drug interactions. Until the manufacturer MAPs’ applications become more standardized and easier to obtain, this method for providing medications will continue to be inefficient. Sustained funding from additional sources (e.g., government or fundraising) is required to serve the indigent populations with prescription medications. Finally, an effort is needed to document the outcomes of drug therapy provided to the indigent and uninsured populations.

Acknowledgments

We appreciate the assistance of Edward Stein, Indian Health Services; Tim Briscoe, George Bachman, and Val Hallman, Grady Health System, Atlanta, Georgia; Gary McWilliams, CareLink, San Antonio, Texas; Marilyn Fortin, Elderly Pharmaceutical Insurance Coverage, New York; Lois Bugos, John Grubbs, and Pam Snyder, Colorado Indigent Care Plan University Hospital, Denver, Colorado; Beth Gerrald, Buncombe County Health Center, Asheville, North Carolina; Health Management Associates, Ingham County, Michigan; Bob McEwan, Medbank, Baltimore, Maryland; and Manju Sankarappa and Ronald Tucker, Prescription for Care, Columbus, Ohio.

REFERENCES


In a double blind, randomised, placebo-controlled, cross-over study 28 children (7-17 years) with exercise-induced bronchoconstriction (EIB) completed two exercise challenge tests (ECTs) 5 min before and 8 hours after a single 12µg formoterol dose delivered via either the Clickhaler® dry powder inhaler (DPI) (Innovata Biomed Ltd) or Foradil® metered dose inhaler (MDI) (Novartis Pharma AG) plus Volumatic® spacer (Allen & Hanburys Ltd). Lung function was measured pre-exercise (baseline), and at 1, 3, 7, 10, 15, 20 and 30 min after each ECT. The primary efficacy variable was maximum % fall in FEV₁ after the 8 hour ECT compared with the pre-exercise value. Secondary efficacy variables were area under % change in FEV₁ against time curve, and time to recover to within 5% of the pre-exercise baseline FEV₁ after the first and 8 hour ECTs. Safety parameters were vital signs, adverse events and tremor. The maximum % fall in FEV₁ (mean ± SD) for the 8 hour ECT was 21.6±16.7 (DPI) and 19.8±14.8 (MDI), with no significant differences between the treatments for this or any other efficacy or safety variable. This study demonstrates clinical equivalence of the DPI and MDI in the treatment of EIB in children.

**Keywords:** Clickhaler, exercise-induced bronchoconstriction, formoterol
INTRODUCTION

Exercise-induced bronchoconstriction (EIB) is an established challenge-based methodology to monitor severity of asthma and evaluate efficacy of asthma therapies (Anderson, 1985; Bisgaard, 2000). It is a particularly beneficial method for use in children because of the speed and objective nature of the test (Remes et al., 2002). Percentage fall in forced expiratory volume in the first second (FEV₁) following exercise challenge, area under the curve (AUC) of % change in FEV₁ against time, and time to recover are commonly used efficacy parameters when comparing inhaled long-acting β₂-agonists by exercise challenge test (Bisgaard, 2000).

A selection of options for delivering drugs to the lung is now available for use in asthma therapy including metered dose (MDI) and dry powder inhalers (DPIs). There are advantages and disadvantages associated with each device such as dose counters, multi dose capabilities, size, and ease of use (Ariyander et al., 1996). When developing a new device for asthma therapy it is important to compare the efficacy and safety of the new device with established inhalers to provide a body of knowledge and choice for the prescriber.

When MDIs are the selected therapeutic option, the additional use of a spacer is considered beneficial, especially for children who find it difficult to use an MDI correctly and who, in particular, find it difficult to co-ordinate device actuation with the inhalation manoeuvre (Pedersen et al., 1986). Use of a spacer can also lessen total systemic exposure to the inhaled drug by reducing oropharyngeal deposition (Newman et al., 1996).

Formoterol, a β₂-selective adrenoceptor agonist with rapid onset and long duration of action, is used as a bronchodilator in patients with obstructive airways disease (Bartow et al., 1998). It is widely available via the Foradil® MDI, the Aerolizer® DPI and the Oxis® Turbuhaler® DPI (Chew et al., 2001). DPIs avoid the use of propellants by utilising the patient’s inspiratory effort to deaggregate the powder into fine particles and to deliver the aerosol into the lung. A dry powder formulation of formoterol is currently in development for use in the Clickhaler®; a bulk reservoir DPI capable of delivering up to 200 doses that has been demonstrated to deliver an effective dose at a range of flow rates (Chege et al., 2000; Newhouse et al., 1999; Warren et al., 1998). The clinical use and patient acceptability of the Clickhaler have been verified previously (Allen et al., 2001; Stradling et al., 2000; Morice et al., 2002).

We used protection against EIB as a method to compare the efficacy and safety of the Clickhaler DPI and the optimally used Foradil MDI plus spacer for administration of single 12µg doses of formoterol to paediatric patients.

MATERIAL AND METHODS

This was a two-centre, double blind, double dummy, randomised, cross-over, placebo-controlled study comprising three clinic visits by the patients (screening, treatment one and two). The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki (1996) and was approved by the local Ethics Committees. Patients or parents/guardians gave written informed consent at the start of the screening visit, which was held 2-21 days before study commencement.

Children aged 7-17 years, with mild to moderate asthma, were eligible to take part in the study if they had an FEV₁ ≥65% of predicted normal and a decrease in FEV₁ of ≥15% following a standardised, validated exercise challenge test (ECT) at screening.

Participation in the study was not permitted if patients had been treated with either oral steroids in the four weeks prior to screening or more than four courses of oral steroids in the past year, or had experienced a respiratory tract infection in the four weeks prior to screening.

If a patient was using inhaled corticosteroids as part of their current asthma medication, the dose must have been stable for four weeks prior to screening. Patients remained on their current asthma therapy outside the treatment visits, apart from short drug washout periods (short acting β₂-agonists 8 hours, long acting β₂-agonists 48 hours) prior to each visit. At each visit, patients used open-label placebo
inhalers for training and to confirm that the inhalers were used according to the manufacturers’ instructions.

Patients were randomised to receive a single formoterol dose (12µg) delivered via either via the Clickhaler® DPI (Innovata Biomed Ltd, UK) or the Foradil® MDI (Novartis Pharma AG) plus Volumatic® spacer (Allen & Hanburys Ltd, Uxbridge, UK). They received one actuation from the inhaler containing the active treatment and one from the alternative placebo device. Patients completed two ECTs: the first 5 minutes before and the second 8 hours after the administration of formoterol. For the screening and two treatment ECTs the children ran on a treadmill with a 10% incline, inspiring dry air through a mask (<25ºC), for approximately 6 minutes at a speed such that a steady-state cardiac frequency of 170-190 bpm was achieved. Lung function (FEV1 and forced vital capacity (FVC)) was measured by spirometry at baseline (pre-exercise), and at 1, 3, 7, 10, 15, 20 and 30 minutes after each test. For patients to remain eligible for study participation pre-exercise FEV1 for each of the ECTs had to remain within 15% of the value recorded at the screening exercise test and above 65% of the predicted normal for FEV1. Following a washout period of 2-21 days, the protocol was repeated with the alternative active treatment and placebo inhalers.

On study days, heart rate was measured by telemetry or electrocardiogram during each ECT and two hours after formoterol administration. Tremor and blood pressure (systolic and diastolic) was measured before the first ECT, and two hours after formoterol administration, with tremor rated on a 0-3 scale where 0 = no visible tremor and 3 = large amplitude, violent jerky tremor. All adverse events were recorded during clinic visits.

Patients’ acceptability of the DPI and MDI was evaluated from answers to questions asked at the end of the second treatment visit. Each randomised patient was asked ‘Which inhaler was easiest to use?’ and ‘Which inhaler do you prefer?’. The investigator was asked to score each device for ease of training and assessment of compliance using a 1 to 5 scale (1 = easy, 5 = hard).

**STATISTICAL ANALYSIS**

A sample size of 26 was calculated using a ratio of mean:SD in FEV1 following ECT, at the 5% significance level and 90% power. The ratio selected for this power calculation was based on data from previously published papers which assessed the efficacy of long-acting β2-agonists on the fall in FEV1 following exercise challenge in paediatric patients (de Benedictis et al., 1996; Boner et al., 1994; Daugbjerg et al., 1996; Henriksen et al., 1992; Nielsen et al., 1997).

All efficacy analyses were completed using the per protocol population, defined as patients with complete data in each treatment period and with no major protocol violation.

The primary efficacy variable was maximum % fall in FEV1 after ECT at 8 hours post-treatment compared with the 8 hour pre-exercise FEV1. The data were assessed using analysis of variance (ANOVA). The DPI and MDI were considered equivalent if the 95% two-sided confidence interval for the difference between the inhalers lay within −10.0% to 10.0%. The secondary efficacy variable of AUC of % change in FEV1 against time after ECT (7-30 minutes post-dose for the first ECT and 0-30 minutes for the 8 hour ECT) was calculated using the trapezoidal rule and also analysed by ANOVA. As this was a secondary variable no equivalence zone (normally 80-125%) was defined. The variable of time taken for FEV1 to return to within 5% of the pre-exercise level following each ECT was analysed using the method of France, Lewis and Kay (1991). A Cox’s proportional hazards model was fitted to the data, stratifying for patient with factors for active inhaler and first/second ECT.

Adverse events and withdrawals were summarised and considered on a case-by-case basis. Vital signs data (systolic and diastolic blood pressure, heart rate) were analysed (ANOVA) comparing pre-dose and two hour post-dose values to evaluate any statistically significant differences between devices. Tremor ratings were summarised and clinically significant values noted. Concomitant medication at baseline and changes during the study period were listed. Patient questionnaires were analysed using the...
RESULTS

Of the 44 children screened for the study 28 were randomised to receive treatments. All patients completed the study procedures. One patient did not match the protocol requirements, having failed to meet the criterion of FEV\textsubscript{1} remaining within 15% of the screened value. The analyses have therefore been conducted using the data from 27 children. Patient demographics, lung function parameters and ECT screening data are shown in Table 1. Unprotected maximum % fall in FEV\textsubscript{1} (mean ± SD (range)) following screening ECT was 36.0±18.1 (14.7 to 67.8) (Figure 1) and was comparable within and between treatment groups. Two patients had FEV\textsubscript{1} values just below 15% but were still randomised to treatment.

Mean ± SD (L) pre-exercise FEV\textsubscript{1} for the first ECT was 3.0±1.0 for the DPI and 3.0±0.9 for the MDI and was 3.2±1.0 and 3.2±1.0, respectively, for the 8 hour ECT. The change in pre-exercise FEV\textsubscript{1} from the first ECT to the 8 hour ECT was similar for both devices.

Table 1: Patient demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clickhaler/MDI (n=13)</th>
<th>MDI/Clickhaler (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>13.5±3.0 (8-17)</td>
<td>14.1±2.5 (7-17)</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/6</td>
<td>8/6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>116.3±12.2 (103-141)</td>
<td>120.6±12.8 (97-141)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>69.4±13.1 (40-89)</td>
<td>70.3±10.2 (54-85)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>83.2±17.4 (56-116)</td>
<td>79.6±15.5 (62-116)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>3.11±1.07 (1.60-5.23)</td>
<td>3.03±0.78 (1.38-4.13)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} as % of predicted</td>
<td>95.2±14.7 (74-125)</td>
<td>90.0±11.4 (69-110)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.68±1.44 (1.92-6.82)</td>
<td>3.59±0.93 (1.53-4.72)</td>
</tr>
<tr>
<td>Maximum heart rate during ECT (bpm)</td>
<td>183.8±4.5 (177-195)</td>
<td>187.2±6.2 (170-195)</td>
</tr>
<tr>
<td>Maximum % fall in FEV\textsubscript{1} post-ECT</td>
<td>41.6±20.4 (16.0-67.8)</td>
<td>32.2±14.8 (14.7-58.3)</td>
</tr>
<tr>
<td>Time to maximum fall post-ECT (min)</td>
<td>7.8±5.3 (3-20)</td>
<td>6.5±4.4 (1-15)</td>
</tr>
<tr>
<td>Time for FEV\textsubscript{1} to return to within 5% of pre-exercise value (min)</td>
<td>25.0±7.4</td>
<td>25.7±7.5</td>
</tr>
</tbody>
</table>
Table 2: Maximum fall in FEV\textsubscript{1} after ECT at 8 hours post-dose with 12µg formoterol for per protocol patients (values are mean ± SD (range))

<table>
<thead>
<tr>
<th></th>
<th>Clickhaler</th>
<th>MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ECT FEV\textsubscript{1} (L)</td>
<td>3.2±1.0 (1.5-5.9)</td>
<td>3.2±1.0 (1.2-5.7)</td>
</tr>
<tr>
<td>Lowest post-exercise FEV\textsubscript{1} (L)</td>
<td>2.5±0.9 (1.0-4.6)</td>
<td>2.5±0.9 (0.9-4.7)</td>
</tr>
<tr>
<td>Maximum fall in FEV\textsubscript{1} post treatment (%)</td>
<td>21.6±16.7 (0-64)</td>
<td>19.8±14.8 (-4-61)</td>
</tr>
</tbody>
</table>

The mean ± SD AUC of % fall in FEV\textsubscript{1} against time was 4.5±11.5 for the Clickhaler and 6.1±10.9 for the MDI after the first ECT, and 12.4±13.2 for the Clickhaler and 10.5±11.1 for the MDI after ECT at 8 hours. No statistically significant differences were found between the devices for AUC of % fall in FEV\textsubscript{1} after the first or the 8 hour ECT.

The median time taken for FEV\textsubscript{1} to recover to within 5% of pre-exercise levels following the first ECT was 10 minutes for the Clickhaler and 15 minutes for the MDI and, after the 8 hour ECT, was 22.5 minutes for the Clickhaler compared with 17.5 minutes for the MDI. There was no statistically significant difference between the inhalers at either time point (Table 3). The number of patients whose FEV\textsubscript{1} did not return to within 5% of pre-exercise challenge level after 30 minutes was similar between the two groups.

Three adverse events were recorded (upper abdominal pain with Clickhaler, wheezing, aggravated dermatitis with MDI), all of which were considered either unrelated or unlikely to be related to study medication. The mean ± SD change in tremor scores between pre- and post-dose was 0.0±0.5 for the Clickhaler and 0.1±0.5 for the MDI. Vital sign and tremor changes were unremarkable and the incidence of clinically notable values was low. These data were not analysed further.

Figure 2a: Mean profile of % fall in FEV\textsubscript{1} following ECT with treatment administration at +5 minutes.

Figure 2b: Mean profile of % fall in FEV\textsubscript{1} following ECT eight hours after treatment.
via the Clickhaler DPI produces similar bronchoprotection to formoterol delivered via the standard MDI plus spacer. The % fall in FEV$_1$ demonstrated following ECT was similar to that seen after treatment with formoterol in four previous EIB studies in children (Boner et al., 1994; Daugbjerg et al., 1996; Henriksen, et al., 1992; Nielsen et al., 1997).

Secondary efficacy data also support the conclusion of clinical equivalence.

This study employed a cross-over design, and the time between treatment visits was minimised to reduce the possibility of any change in the patients’ asthma status. To ensure that there was no drug carry-over effect, a minimum period of 48 hours was allowed between the two treatment visits, as recommended by the American Thoracic Society Guidelines for Methacholine and Exercise Challenge Testing (American Thoracic Society, 1999).

Most studies of long acting β$_2$-agonist bronchoprotection use peak protection (e.g. 1 to 2 hours post dose) to assess the effects of the drug, even though the duration of action is up to 12 hours. The period used to consider the long acting effects of formoterol in this study was 8 hours post dose. This represents a realistic time-point that gives both valid data and a practical length of study day for the children participating.

Previous studies indicate that formoterol can be utilised as an effective rescue medication for the immediate relief of asthma symptoms (Grembiale et al., 2002; Ferrari et al., 2002) and ‘as needed’ use of formoterol is now recommended for patients with moderate asthma who are also taking glucocorticosteroids (Global Initiative for Asthma, 2002). Although the focus of this study was to evaluate the prophylactic effect of formoterol following the 8-hour ECT, we were also able to assess the immediate effect of formoterol following the first ECT. The number of patients with an FEV$_1$ returning to within 5% of pre-exercise levels (within 30 minutes of ECT) was >80% following the first ECT, >60% following the 8 hour ECT compared

**DISCUSSION**

When assessing the comparability of inhaler devices, it is imperative to use valid methods that demonstrate relevant treatment effects (Buck, Parry-Billings, 2001). We used the established methodology of EIB, which measures the effect of short and long acting bronchodilators, such as formoterol, on airway hyperresponsiveness in asthma patients (Britton, 1998). Other challenge-based tests to measure hyperresponsiveness in asthma are available, for instance, those using bronchoconstrictor agents such as methacholine, histamine, and adenosine monophosphate (Sterk et al., 1993) We chose exercise challenge testing rather than graded doses of these bronchoconstrictor agents as the more practical method for use in paediatric studies (Britton, 1998). This sample was sufficient to detect a difference of one SD between treatments in the maximum % fall in FEV$_1$ following ECT 8 hours after treatment with 12µg formoterol delivered via a DPI and MDI. The 95% confidence interval for the difference between inhalers was within –10 to 10%, indicating that, in paediatric patients with EIB, formoterol delivered

| Table 3: Time taken for FEV$_1$ to return to within 5% of pre-exercise challenge level after ECT for per protocol patients |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| First ECT (7-30min) | 8-hour ECT (1-30min) |
|                 | Clickhaler | MDI | Clickhaler | MDI |
| Number returning | 22 (81%) | 24 (89%) | 17 (63%) | 18 (67%) |
| Number not returning | 5 (19%) | 3 (11%) | 10 (37%) | 9 (33%) |
| Median (mins) | 10.0 | 15.0 | 22.5 | 17.5 |

The DPI was significantly favoured by the patients compared with the MDI (p<0.001): 82% preferred the DPI, 11% preferred the MDI and 7% had no preference. In addition, 68% considered the DPI easier to use compared with 11% who found the MDI easier to use, while 21% had no preference. There were no statistically significant differences between inhalers in terms of the investigator scores for training or compliance assessment. For ease of training, the mean ± SD score was 1.3±0.7 for the Clickhaler and 1.1±0.6 for the MDI; for compliance assessment the score was 1.2±0.4 for the Clickhaler and 1.1±0.5 for the MDI.
with 26% following the screening ECT. This demonstrates that formoterol provided an immediate treatment effect following the first ECT, and a prophylactic effect at the 8 hour ECT.

Patient acceptability of the Clickhaler was high: most children found it easier to use than the MDI plus spacer, and acceptance of a device may have an impact on compliance in the clinical setting. Children (Parry-Billings et al., 2003), and many aged from 3-5 years (Iqbal, et al., 2003) are able to generate adequate inspiratory flow to operate the Clickhaler effectively. These findings are supported by recent therapeutic studies showing clinical efficacy equivalent to MDIs (Adler et al., 2001). In addition, the incidence of adverse events was very low indicating no evidence of any safety issues associated with formoterol treatment via Clickhaler DPI or Foradil MDI in these paediatric patients.

Formoterol produces bronchodilatation for at least 12 hours when the recommended dose of 12µg is administered (Lecaillon et al., 1999) and for this reason long-acting β₂-agonists such as formoterol may be preferable to short-acting bronchodilators in terms of compliance, especially amongst children. As long-acting β₂-agonists become the preferred therapeutic option over increased corticosteroid doses for children over 5 years of age, (British Thoracic Society, 2004) it is important to provide a wider choice of delivery options for drugs such as formoterol.

Acknowledgements

The expert assistance of Laura Baines in the preparation of this manuscript is gratefully acknowledged.

REFERENCES


Oxaliplatin and targeted adjunct-chemotherapy for bowel cancer through DNA-binding: causal involvement of ROS?

ALAN WISEMAN AND PETER GOLDFARB

School of Biomedical & Molecular Sciences, University of Surrey, Guildford, GU2 7XH

Correspondence: Alan Wiseman, School of Biomedical & Molecular Sciences, University of Surrey, Guildford GU2 7XH
email: alan@tridgeway.wanadoo.co.uk

Oxaliplatin is a novel drug that acts against bowel cancer, especially in combination with other well known chemotherapeutic agents. Its mode of action is through binding to DNA.

There is the question as to whether reactive oxygen species (ROS) is involved in the causation of bowel cancer due to the irritation of bowel tissues associated with such diseases as Crohn’s disease.

Keywords: Oxaliplatin, chemotherapy, platinum, bowel cancer, ROS (reactive oxygen species)
Oxaliplatin binds to DNA in tumours and modulates protein-enzyme interactions including replication of the DNA and transcription (Coulson, Wiseman and King, 1984; Coulson, 1994; Lewis, 2001; Grothey, and Goldberg, 2004) to produce the messenger RNA available for translation (expression) into proteins including enzymes (Cheney, Campbell, Temple et al 2004; Samini, Manrock, Castel et al. 2004; Andre, Boni, Moundeji-Boudiaf et al. 2004; Guglielmi, Barni, Zaniboni, 2004). Oxaliplatin has been reported to be effective in adjuvant treatment with fluorouracil and leucovorin (Andre, Boni, Moundeji-Boudiaf et al. 2004) also with methotrexate and fluorouracil (Guglielmi, Barni, Zaniboni, 2004). Moderate remissions were observed, along with mild-symptom side-effects related to toxicity of these multidrug regimes. Possible generation of some reactive oxygen species (ROS) should be subjected to more investigation in further adjunct-chemotherapeutic evaluation of oxaliplatin efficacy! (see below).

METALS IN ROS GENERATION

Metals in benemins (hormeners) possess good-health potential in “minimal-dose”, but can be toxic at much above this daily dose (Calabrese and Baldwin, 2003). For example, in the case of dietary selenium intake, this metalloid mineral is essential at 50 micrograms/day because of its incorporation into glutathione peroxidases (this destroys peroxide, -O_2^-). Nevertheless, dietary selenium is toxic at 500 micrograms/day, its benemin index (BI) being 10 (500/50) therefore (see Table 1).

Toxicity (Halliwell and Gutteridge, 1999) is associated with biomolecular free radical damage (Wiseman, Goldfarb, Ridgway et al. 2000; Abele, 2002). Indeed, oxygen itself is a rather toxic effector of cellular biometabolism due to the formation of reactive oxygen species (ROS).

Some 4500 isoforms of cytochromes P450 contain iron in protohaematin IX (see below). Mimics are being sought that contain ruthenium complexes other than with the redox metal iron held within a porphyrin prosthetic group therefore.

**PRODUCTION OF ROS BY CYTOCHROMES P450**

Harmful free radicals, such as superoxide anion (-O_2^-), are often produced in the body during aerobic respiration in all tissues, because of only partial reduction of some oxygen molecules in the mitochondrial respiratory chain (via cytochrome

| Table 1. Biomolecular basis for a benemin-index (BI) for dietary metals |
|------------------|--------------------------|--------------------------|
| **Dietary metal ion damage** | **Low dose benefit via enzymes** | **High dose toxicity via biomolecular damage** |
| Selenium | Antioxidant (50µg/day) (glutathione peroxidases) | Toxic (500µg/day) BI=10 (500/50) |
| Iron (II)/Iron (III) | Enzyme cofactor | Pro-oxidant |
| Manganese | Enzyme cofactor in mitochondrial superoxide dismutases | ? |
| Cobalt | Component of vitamin B12 | ? |
| Chromium | Anti-diabetic claim | Chromium picolinate may be toxic |
| Vanadium | Anti-diabetic claim | ? |
| Copper | Enzyme cofactor in cytosolic superoxide dismutases | Pro-oxidant |
| Zinc | Enzyme cofactor: and in Zn-finger protein (DNA-regulating). Immune system stimulant | ? |
| Sodium | Nerve action Z potential: and lipoprotein channels and gates: Na+/K+ ATPases: intracellular signalling systems | Kidney damage (leading to hypertension) |
oxidase) redox metals that include iron (Riedl, Shamsi, Anderton et al, 1996). This is due to one-electron reduction of each atom of oxygen. Normally the terminal electron acceptor O₂ undergoes overall a four electron process when combining O₂ with 4 H⁺ to give H₂O. Cytochromes P450 (EC 1.14.14.1 O₂: mono-oxygenase CYP) are found in 4500 isoforms (57 in humans). These employ an electron transfer chain from NADPH to water (with an insertion also of one oxygen atom into xenobiotic substrates) that uses this mixed function oxidase family (Lewis, 2001) as the terminal electron acceptor. Nevertheless, these enzymes and their alternative-metal mimics may display futile cycling of electrons at low concentrations of substrate, to produce ROS in the form of superoxide anion (·O₂⁻).

AVOIDANCE OF DIETARY PRO-OXIDANTS DURING CHEMOTHERAPY

The lowering of ROS levels is a priority in the avoidance of particular diseases now identified (Halliwell and Gutteridge, 1999). For example, damage to biomolecules can occur after attack on phospholipid membranes that produces hydroperoxides that break down to form ROS especially in the presence of iron-catalysed Fenton chemistry. Proteins are either antioxidant or pro-oxidant in the TBARS-formation test (by preventing or promoting the formation and subsequent breakdown of peroxidised lipids to malondialdehyde – see Table 2). This redox position is determined by amino acid residue composition and stability (Riedl, Shamsi, Anderton et al, 1996). Moreover, some isoforms of cytochromes P450 are likely to be pro-oxidant also in human food bioprocessing where their deployment may be ill-advised therefore, to avoid the consumption of oxidised foodstuffs during chemotherapy.

By definition, useful antioxidants are readily oxidised; especially by pro-oxidants. For instance, ascorbic acid (vitamin C) loses two hydrogen atoms in conversion to dehydroascorbate (which is formally a free-radical weak pro-oxidant). Ascorbic acid is an acidic enol (sugar-derived) that is converted upon oxidation (in vivo, and by heating or prolonged storage) into the dehydroascorbate (by removal of the two hydrogen atoms that are otherwise ionizable, and thus can display acidic properties in solution). This makes ascorbic acid a powerful reducing agent commonly employed in regeneration of iron II (from iron III) for the prolonged production of free radicals in vitro from the phospholipids of ox-brain liposomes when assayed by TBARS production (Halliwell and Gutteridge, 1999) (see Table 2). Nevertheless, in vivo, vitamin C is an essential component of the human diet being required daily and contributing to the protection

<table>
<thead>
<tr>
<th>Table 2. Detection of products of biomolecular damage caused by ROS.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIOMOLECULE SUBJECT TO DAMAGE BY ROS</strong></td>
</tr>
<tr>
<td><strong>Phospholipid</strong></td>
</tr>
<tr>
<td>1. Production of malondialdehyde: detected with thiobarbituric acid (pink colouration read at 532 nm).</td>
</tr>
<tr>
<td>2. Isoprostane assay (GC-MS or HPLC)</td>
</tr>
</tbody>
</table>

HDL is High Density Lipoprotein
LDL is Low Density Lipoprotein
(both HDL and LDL carry cholesterol in the blood stream)
GC-MS is gas chromatography-mass spectrometry
HPLC is high performance liquid chromatography
NMR is nuclear magnetic resonance spectrometry
Oxaliplatin and targeted adjunct-chemotherapy for bowel cancer through DNA-binding

of the body from ROS, along with membrane-soluble vitamin E and a variety of other plant-derived antioxidants such as tea polyphenols (quercetin and others) and the soy phytoestrogen isoflavones (daidzin and genistin are the glucoside forms) (Wiseman, Goldfarb, Ridgway et al. 2000) – see Table 3.

### ANTIOXIDANT : PRO-OXIDANT MOLECULAR-COUPLES OF METALS IONS

The standard redox potential ($E_0^1$) for the molecular oxygen:water couple is +0.815 volts, as measured with an appropriate electrode. All values quoted for $E_0^1$ refer to the system in the presence of equal concentrations of the oxidised and reduced forms (as specified by the Nernst equation); and any departure from this norm will result in a corrected value ($E_o$) that can differ very markedly from $E_0^1$. In vivo, therefore, assumption should not be made a priori from $E_0^1$ as to the direction (or extent) of electron flow along a postulated sequence of electron donors/acceptors (which alternate between reduced and oxidised forms).

A simple example to consider is the interconversion of iron(II) and iron(III): iron II ($\text{Fe}^{2+}$) loses one electron during its oxidation to the iron III ($\text{Fe}^{3+}$) form and thus these two forms of iron are antioxidant ($\text{Fe}^{2+}$) and pro-oxidant ($\text{Fe}^{3+}$), respectively. Although cytochromes P450 (see above) have this couple (Samini, Manrock, Castel. 2004: their mechanism of action progresses through ironII/ironIII/ironV. The iron perferryl is thought to occur in the oxonium intermediate $[\text{FeO}]^{3+}$ reached from previously formed FeII/$\text{O}_2$// peroxide

<table>
<thead>
<tr>
<th>Phytoproducts</th>
<th>Indications</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine (in coffee and in tea-infusions)</td>
<td>Stimulant needed</td>
<td>Nervous system can be overstimulated</td>
</tr>
<tr>
<td>Phenylamines and theobromine in cocoa/chocolate</td>
<td>Mild “depression”</td>
<td>Overstimulated-brain syndromes</td>
</tr>
<tr>
<td>Phytoestrogens: these nutraceuticals in soya are genistin and daidzin the polyphenolic glucosides: these are oestrogen agonists and also display membrane antioxidant ability</td>
<td>Menopausal symptoms. Also in prevention of osteoporosis, coronary heart disease, breast cancer. Possible anti-ageing and cosmetic applications</td>
<td>Male neonates especially, merit a cautious approach: as with xenoestrogens in the environment</td>
</tr>
<tr>
<td>Brewers' yeast and baker's yeast (Saccharomyces cerevisiae)</td>
<td>Vitamins B dietary deficiency</td>
<td>Tough yeast cell wall indigestibility limits the daily dose tolerated</td>
</tr>
<tr>
<td>Vitamin C 70-200 mg/day (from citrus fruit) is the normally recommended daily intake (RDA)</td>
<td>Antioxidant (spares vitamin E): and immune system stimulant</td>
<td>May require acidity regulation (at 1-3 g/day) such as by use of the calcium or sodium salt of ascorbic acid</td>
</tr>
<tr>
<td>Vitamin E perhaps 10-100 mg/day (from wheatgerm)</td>
<td>Membrane antioxidant against oxidative stress and ROS</td>
<td>Macrophages produce ROS in respiratory-burst attack on bacteria in the bloodstream: excessive antioxidant accumulation could oppose this benefit</td>
</tr>
</tbody>
</table>

**Key**

ROS = reactive oxygen species  
Vitamin E = $\alpha$-tocopherols  
Vitamin C = ascorbic acid

Note: genistein and daidzein (the aglucone forms of genistin and daidzin respectively) are formed by hydrolytic cleavage by bacteria in the gut.
intermediate (see on) that derives from the FeIII/substrate complex formed initially in the mechanistic cycle of all iron-containing cytochromes P450. It is the FeIII form that initially binds the substrate (R) and this is reduced to FeII.R(O2) by an electron supplied via NADPH and cytochrome P450 reductase (a flavin) (see on).

It may seem paradoxical therefore that Fe3+/ascorbate is commonly employed to generate Fe2+ (ostensibly the antioxidant form of the iron III: iron II couple) to catalyse the breakdown of phospholipid liposomal membrane model-systems. Fe2+, however, is the form that reacts with lipid hydroperoxides (ROOH) that have accumulated in the ox-brain test system phospholipid mixture during storage in the atmosphere, to form peroxyl (ROO·) and alkoxyl (RO·) derivatives of phospholipids (Riedl, Shamsi, Anderton et al. 1996). These free radicals initiate a chain reaction of further attack of the phospholipids via degradation reactions that produce large amounts of these free-radical species (Halliwell and Gutteridge 1999). Chain-breaking antioxidants acting in the lipid phase include vitamin E. This therefore is an important protective agent in the diet that terminates unhealthy propagation of the lipid-derived free radicals that are thought to cause (or to be associated with) many diseases (Wiseman, Goldfarb, Ridgway, 2000; Wiseman 1994). It is vitamin E (lipid phase) and vitamin C (aqueous phase) together that provide the major functional component of the dietary antioxidant nutraceuticals necessary for combating oxidative stress in humans, especially appropriate in bowel cancer prevention and chemotherapy.

Potentially toxic metals often display one-electron recycling within the framework of dual (or multiple) valency-state abilities. For example, this occurs between FeII and FeIII (see below), also CuI and CuII: other possibilities include electron cycling between FeIII and CuII or FeII and CuI and other combinations of redox metal ions (see Table 1). Platinum is less likely to achieve such one electron recycling.

In mechanistic utilisation of two electrons and oxygen in cytochromes P450 (see Table 4), the iron atom FeIII in the protohaematin (IX) porphyrin complex (Lewis, 2001) firstly binds the substrate (R). After binding of the substrate on to FeIII, reduction of the FeIII(R) to FeII(R) can proceed (see above), and this FeII(R) takes up O2 to form the Fe(II)RO2// peroxide complex. Next [FeO]3+ is formed, where the iron oxonium cation is in the perferryl valency state (FeV) – the second electron required is supplied now by cytochrome b2. In this phase I biochemistry mainly in the liver, the oxygen of this mixed function oxidase (oxidogenase) is thereby split, with products H2O (it is 2H+ that have combined with an oxygen atom) and one oxygen atom entering the substrate (RH), after heterocyclic removal (and replacement) of a hydrogen atom (Lewis, 2001). This reaction sequence forms the more hydrophilic product ROH (this may be conjugated with glucuronic acid in the phase II biochemistry prior to excretion through the kidney).

It follows that antioxidant/pro-oxidant coupling should be recognised as a cause of toxicity of alternate valency-states in a metal or mixture of metals; one antioxidant, the other pro-oxidant. This is especially necessary where one electron cycling by redox metals can unfortunately generate ROS: when substrate levels are low. A cautious approach is recommended where metals have been introduced into complexes such as in oxaliplatin.

MULTIFUNCTIONAL-FOOD AS PROTECTANTS IN CHEMOTHERAPY

“Multifunctional food” (nutraceuticals) is a descriptive label used usually to suggest beneficial features not possessed by merely “physiological food” eaten mainly to provide metabolic daily energy and necessary bodily-repair. For instance, unsaturated fats and oils, especially those containing polyunsaturated fatty acids (PUFAs), have many other beneficial roles, now being identified; but they require more dietary antioxidant vitamin E to limit the possible formation of unhealthy free radical fragments.

Vitamins and minerals are “non-nutrient” dietary components, recognised to be mainly enzyme-cofactors (or prosthetic groups) (Lewis, 2001; 15. Wiseman, 1994; Wiseman, Ridgway and Wiseman, 1999; Wiseman, 1995; Tucker and Woods, 1997; Wiseman, 1993) in their final form (after minor modification in the body). For example, vitamin B1 (thiamine is a component of thiamine pyrophosphate (TPP) carboxylase in the conversion
Oxaliplatin and targeted adjunct-chemotherapy for bowel cancer through DNA-binding

of pyruvate to acetyl CoA, for use in the Krebs tricarboxylic acid cycle: all the fat-soluble vitamins (A,D and E) are now recognised to be antioxidants (Gamble, Wiseman and Goldfarb, 1997; Wiseman, Ridgway, Goldfarb and Woods, 2002) as is selenium (see Table 1) (Rayman, 2002). Moreover, several polyphenolic compounds of plant foods, such as soya, are antioxidants (these include the isoflavone glucosides daidzin and genistin: see Table 3).

Although benemin-index (BI) (see Table 1) may become the accepted benchmarking criterion for some components of food (but beware of “overdetection” of toxicants (Wiseman, Ridgway, Goldfarb and Woods, 2002), the fine line between multifunctionality and dysfunctionality may be cryptic when based upon criteria such as age, gender and state of health (and lifestyle habits) (Rayman, 2002; Wiseman and Woods, 2002; Brown and Sassoon, 2002; Opara, 2002). Moreover, the emerging data on idiosyncratic responses, including hypersensitivities and allergies, should be viewed now in the wider context of an individual’s nutragenomics gene-profiling data: and their much more complex proteomic profiles that together allow the bioinformatics approach.

### Table 4. Some of the 4500 cytochromes P450 (CYP) isoforms (57 in humans). All of these CYP isoforms contain protohaematin IX iron; but CYP mimics may contain other redox-metal centres (such as ruthenium complexes) (*)

<table>
<thead>
<tr>
<th>Cytochromes P450 isoform</th>
<th>Natural sources (animal, plant or microbial)</th>
<th>Substrate (mode of biotransformation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Liver; lung (mammalian)</td>
<td>Benzo(a)pyrene (hydroxylated into carcinogenic forms)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Liver (mammalian)</td>
<td>Testosterone (hydroxylated)</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Liver (mammalian)</td>
<td>Ethanol (oxidised to ethanal in free radical mechanism)</td>
</tr>
<tr>
<td>CYP51</td>
<td>Candida lipolytica</td>
<td>Alkanes (ω-oxidised)</td>
</tr>
<tr>
<td>CYP51</td>
<td>Candida tropicalis</td>
<td></td>
</tr>
<tr>
<td>CYP51</td>
<td>Saccharomyces cerevisiae</td>
<td>Lanosterol (14α-demethylated)</td>
</tr>
<tr>
<td>CYP450&lt;sub&gt;DM&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP101 (CYP450&lt;sub&gt;CAM&lt;/sub&gt;)</td>
<td>Pseudomonas putida bacteria</td>
<td>Camphor (5-hydroxylated to borneol)</td>
</tr>
<tr>
<td>CYP74</td>
<td>Arabidopsis thaliana</td>
<td>Alkane oxide synthase (hydroperoxide lyase)</td>
</tr>
<tr>
<td>CYP73A6-8</td>
<td>Zea mays</td>
<td>Cinnamate 4-hydroxylase</td>
</tr>
<tr>
<td>CYP85</td>
<td>Lycopersican esculatum</td>
<td>Gibberellin biosynthesis</td>
</tr>
</tbody>
</table>

(*) CYP mimics could be constructed using platinum complexes with other metals such as iron and ruthenium.

**METAL-CHOICE IN ADJUNCT-CHEMOTHERAPY**

A safe choice of metals must be given priority in adjunct-chemotherapy. Furthermore enzyme-mimicry is proposed where circumvention of pH- and thermal-incompatibility is sought in multienzyme use (Wiseman and Woods, 2003; Wiseman, Lewis, Ridgway and Wiseman, 2000; Wiseman and Woods, 2002). In addition, novel enzymes can be generated in micro-organisms by forced (directed) evolution (Wiseman, Goldfarb, Woods and Ridgway, 2001). Here cytochromes P450 mimics that emerge may utilise metals other than iron; according to the growth media composition used to evolve such necessary enzymes under harsh growth conditions. Utilisation of enzymic bioprocessing of human foods will increasingly require real-lab testing and *in silico* toxicity predictions (Wiseman, 2003): and subsequent avoidance. Nevertheless, especially chronic toxicity is difficult to extrapolate from heterogeneous populations to individual-avoidance recommendations (Wiseman, 2003; Wiseman, 2003; Wiseman, 2003; Wiseman and Woods, 2001). Nutragenomics from 30,000 genes per human cell and the much more complicated proteomics (150,000 proteins per human cell) will
result in a wide spectrum of dietary antioxidant requirements. Herein is the basis of an individual’s strategy of the healthy avoidance of oxidative stress mediated by ROS (Halliwell and Gutteridge, 1999) and by other harmful free radicals such as reactive sulfur species (RSS) such as is found in the alkylsulfur free radical (·SR) (Riedl, Shamsi, Anderton, Goldfarb and Wiseman, 1996).

Avoidance of food-generated free radicals will become of increasing importance (Halliwell and Gutteridge, 1999; Wiseman, Goldfarb, Ridgway and Wiseman, 2000; Abele, 2002) and food-bioprocessing itself will need biomonitoring (Wiseman, 2003; Wiseman, 2003). Nevertheless, enzyme mimics with alternative metals will reflect the situation in cell biology, where for instance the superoxide dismutase of mitochondria contains manganese, whilst those of the cytosol contain copper/zinc (bacterial forms often contain iron, although copper/zinc isoforms are present in some bacteria).

**CYTOCHROMES P450 REDOX METAL MIMICRY**

For example, the well-documented toxicity of selenium (selenosis) at about 500 micrograms per day in the food and water consumed daily (Rayman, 2002) necessitates an urgent programme of research to be initiated.

Selenium resembles sulfur, having six electrons in its outer shell, but unlike sulfur, it has a large cloud of electrons that is readily distorted: its ability to give or take electrons determines its characterisation as a metalloid (Rayman, 2002) (the energy difference band gap between ground state and excited states is only 2 eV). It is of interest that selenium is the only metal to have its own genetic code; employed for incorporation of selenocysteine into proteins (Gamble, Wiseman and Goldfarb, 1997) (uracil-guanine-adenine are the bases in the triplet codon messenger RNA sequence for the incorporation of selenocysteine into proteins (Gamble, Wiseman and Goldfarb, 1997; Wiseman, Ridgway, Goldfarb and Woods, 2002; Rayman, 2002; Wiseman and Woods, 2002; Brown and Sassoon, 2002).

Analogies with toxic-oxygen and toxic-sulfur may suggest a free radical causation, much as with ROS and RSS: indeed, in selenosis-causation can RSeS (reactive selenium species) be ruled out of the toxicity indictment?

Appraisal of alternative metal-containing mimics of cytochromes P450 would require a metal that can mimic iron closely. The Periodic Table in the same group to iron indicate that ruthenium is the obvious choice that indeed has been investigated in porphyrin or phthalocyanine complexes (Fajan’s rules can be invoked to predict the behaviour of ruthenium compared to iron – see on).

The ruthenium nucleus has looser control over peripheral electrons (much like the comparison of selenium with sulfur above) and appropriate application of Fajan’s rules therefore will illustrate the increased flexibility of the electron cloud in the ruthenium complexes. Nevertheless, although candidate-substrate binding is achieved, the catalytic constant (kcat/Km) is low. Indeed, it is low in several iron-containing regular isoforms of cytochromes P450. This consideration includes the CYP51 (see Table 4) isolated from Saccharomycyes cerevisiae that is employed here as a lanosterol 14α-demethylase in the bioconversion pathway to ergosterol (analogous to the cholesterol biosynthesis pathway in animals, including humans).

Nevertheless, in silico investigation may be inadequate in predicting high catalytic constants for alternative metal mixed function oxidases and real-lab studies will need to be pursued where CYP mimicry is intended. Moreover, chronic toxicity cannot be ruled out without thorough investigation of bioavailability both in bioprocesses and in the body (Wiseman, 2003; Wiseman and Woods, 2001). No doubt, further investigations on platinum and other metals will improve the clinical outcomes of the adjunct chemotherapy.

Furthermore, it is of considerable interest that Crohn’s disease of the bowel is thought to be due to inflammation of the bowel triggered by ROS. Moreover, this condition can be a precursor to bowel cancer development in some patients.
REFERENCES


Guide for authors

A. Presenting the material
Original articles of up to 3000 words (4 to 10 printed pages) are invited for publication and should be sent to the Editor in Chief or the Regional Editors. Articles will be reviewed by two referees with a rapid decision for acceptance and subsequent publication. Review articles of up to 8000 words (12 printed pages) may be published in each issue at the invitation of the Editor in Chief but authors wishing to submit such articles should first send a summary to the Editor in Chief before starting detailed work.

Submission: Original articles should be sent to:
Europe: David Luscombe, Editor in Chief, Welsh School of Pharmacy, University of Wales, King Edward VII Avenue, Cardiff CF1 3XF, UK
USA: Jerry Bauman, College of Pharmacy and Medicine, University of Illinois at Chicago, 833 South Wood Street, m/c 886, Chicago, Illinois 6012-7230, USA
Japan: Hiroshi Okada, Department of Hospital Pharmacy, Aichi Medical University, Nagakute-cho, Aichi-ken 480-1195, Japan.

The title page should include the title of the paper and the name(s) and position(s) of the author(s). Indicate the principal author where relevant. Also supply the name, contact address and daytime telephone and fax numbers of the person who will check the proofs.

Each paper requires an abstract of up to 200 words, presented on a separate sheet of paper. Each paper must also have up to six keywords for indexing and data retrieval purposes.

Submit your text on a 3 1/2-inch disk or as an e-mail file (preferably in Microsoft Word) with one hard copy printout, and include any illustrations. Please include any original data used to draw a graph. The printout should be formatted; in other words it may contain stylistic devices such as bold, italic underlined text, superscripts and subscripts.

Label the disk clearly with your name, the title of your paper and the software program (for example, Microsoft Word 2000, etc).

B. Preparing the text
In preparing the text, only use the space bar to provide space between words. For multiple spacing, use the tab command.

The text should be divided into sections and given plentiful sub-headings, but avoid section numbering.

Abbreviations and acronyms should be spelt out in full the first time they appear within the text and, if necessary, defined.

C. References
These should be cited using the Harvard system. References are indicated in the text by authors' names (et al. should be used for papers authored by more than two people) and year of publication. The full list should be collated alphabetically at the end of the paper. References should follow the style below.

Journal articles

Books

D. Handling illustrative material
Indicate within the text where figures and tables should fall.

If graphs or tables are presented as an integral part of the text, they should be treated as a self-contained addendum section at the end, following the references and numbered according to the order in which they appear in the text.

The source of all graphic or tabular material must be provided.

Legends for diagrams supplied separately should also be keyed into this addendum, as should captions for photographs. The matching number should be lightly written in pencil at the top right-hand corner of diagrammatic material.

Nothing should be written on photographs. Instead, write the matching reference number on a separate sheet, as well as arrows indicating which is the correct way up in use.

35mm slides are acceptable and photographs should be glossy, good contrast prints in black and white or colour, ideally at least 150mm x 100mm in size. If supplied electronically we need the original picture file in either a TIFF or EPS format and not just embedded within the text.

Good quality line drawings are also acceptable, and should include all the relevant details. If facilities for producing line drawings are not available to the author, figures can be redrawn by the publishers.

All material should be sent to the Managing Editor, Joe Ridge, Euromed Communications Ltd, The Old Surgery, Liphook Road, Haslemere, Surrey GU27 1NL, UK. Electronic files should be sent by e-mail to: joe.ridge@euromed.uk.com

Your disk and photographs will be returned soon after publication.

E. Proofs
Authors will receive page proofs (including figures) for correction, which must be returned within 48 hours of receipt.

F. Reprints
Ten reprints will be sent to the corresponding author free of charge. Additional reprints may be ordered by completing the appropriate form sent with proofs.

Thank you for your co-operation.