

WORLD CONGRESS OF PHARMACY AND PHARMACEUTICAL SCIENCES

The human genome: a brave new world of chemical proteomics

Our final set of highlights from the recent Nice congress of the International Pharmaceutical Federation includes reports of a symposium on building in safety during drug development and on the importance of standardisation techniques for herbal medicines



A symposium entitled “Building in safety during drug development”, jointly organised by the Industrial Pharmacy section and the Laboratories and Medicines Control Services section, was held on 5 September. It examined the opportunities for the pharmaceutical industry more quickly and efficiently to develop innovative new drugs to treat unmet medical needs.

In her introduction, chairman Dr Linda Hakes (Schwarz-Pharma, Germany) noted that regulators, patients and the health care professions have variously expressed concern that safety might be compromised if new medicines are developed and approved too quickly. This fear could be allayed by the use of modern scientific methods and technologies to reduce the time needed to bring innovative new medicines to the market while maintaining the highest standards of safety.

UNLOCKING THE PHARMACOLOGY OF THE HUMAN GENOME

In a presentation of computer-driven video-clips portraying virtual behaviour of prospective molecules and active sites, Dr David Bailey (Purely Proteins Ltd, Cambridge, UK) described the United States and European joint publication in 2001 of the first draft statement of the structure of the human genome as “just the beginning of a voyage of discovery”. Over 50 per cent of the “genomic landscape is still uncharted biochemical territory” and he observed that of almost 5,000 current drug “targets” identified by molecular mechanistics, less than one fifth fall into known “pharma” territory.

Dr Bailey presented a thematic integration of discovery technologies leading to a drug candidate. It seems that the traditional sieve of pharmacology screening is multiplying dramatically and, he suggested, the majority of future drugs will be large biomolecules, while much of the remaining small molecule research would increasingly be driven by computers. Time to development for lead drug candidates is already near optimum and is not expected to get much shorter, he said.

Taking the topical example of development of HIV protease inhibitors, he proceeded to identify mechanisms to improve understanding of three key discovery endpoints: targets, leads and candidates. Using elegant video-clips, he portrayed structured design of HIV as the most highly researched protein in the world and related it to metallo-, cysteine- and serine-subfamilies of proteases. His interactive 3-D computer models facilitated prediction of selectivity of sites and he speculated “what would happen if” a specific drug structure were to enter them. Fine mapping of sites and associated ligands is essential for accurate definition of available “pockets”, side chain flexibility at site, and to construct feasible drug scaffolds. However, as a Roche 3D screening program had shown, despite good binding and (seemingly) less drug molecule movement, the scaffold would still predict a million potential molecules. He demonstrated virtual screening of existing compounds, matching fragments and complete molecules to specified sites, with energy and volume criteria for optimum binding. The docking procedure revealed how, over a range of targets, candidate molecules might “learn” to fit into sites by “stochastic tunnelling”. Such sophisticated software could assess binding efficiency, identify new receptors and compare superimposed structural features, at possibly “unknown receptors and uncharted binding sites”. He called this “chemical proteomics”.

Proceeding from the prediction of candidates at virtual sites, he came to the molecular synthesis phase. He identified the “chemical drivers” as combinatorial chemistry, high throughput parallel analysis and equipment miniaturisation. These drivers have served well for small molecules but molecules of the new era are much larger and it is, said Dr Bailey, “necessary to engineer selectivity” on a full scale running from low molecular weight to large, up to protein and, ultimately, to cellular level.

In the final section of this keynote presentation, Dr Bailey identified the “key technology hurdle” — data integration. He speculated that chemical design might present 140,000 design strategies for an average

site with 10,000 scaffolds per strategy, examined in 100-member combinatorial “libraries”, accounting for 1,011 possible enumerated compounds. In a screening experiment with 100,000 human genes per chip, 10 time points each with 10 doses and 10 replicates would collectively generate 108 data points per experiment.

What more then in this “brave new world”? With 300,000 proteins and 30,000 genes, “would there be a drug for every gene?” he asked. “No!” There are too many issues of selectivity and multiple sites — and redundant functions. But quite possibly there would be drugs to suit a large number of genes. “Chemical proteomics will tell us soon,” he said. This, together with ligand design, held the key to design many new, safe, drugs to flow from the human genome project.

MODERN TECHNOLOGIES IN ANALYTICAL PROFILING

Professor Bill Dawson (Proteome Sciences, UK) noted that analytical specifications for new medicines have “changed dramatically over the past decade or so”. With so many biotechnology products entering clinical trials, “technologies that were previously in the protein scientists’ domain have been added to the analysts’ armamentarium”. “Complex analytical equipment,” he went on, “has become user-friendly and more affordable.” Concurrently, computer power has increased beyond [all historic] recognition and the power of relational databases has provided the capacity for handling information from many data sets.

Classical spectroscopic techniques have been refined, and new computational techniques incorporated here, too, which has increased both sensitivity and turn-round times. He instanced the advantage of near infrared (NIR) spectrometry for non-invasive identification of solids: the instru-

ments are relatively cheap (*ca* £50,000) and can also be used online in production. To demonstrate NIR versatility, Professor Dawson displayed some 2D orthogonal plots that provided discrimination of species of digitalis and the geographic origin of cannabis seizures.

Sophisticated separative technologies have also evolved, such as robotic driven 2D electrophoresis. Many traditional separative systems are now routinely coupled with a variety of output instruments, notably bench-top mass-spectrometers (MS) but also with nuclear magnetic resonance, ultraviolet and infrared spectrometers. Equipment miniaturisation has facilitated examination of sub-microgram quantities, eg, a nano-scale high performance liquid chromatography system coupled to time-of-flight (TOF) MS, and sensor assemblies incorporating immunological reagents on novel supports.

Professor Dawson stressed that such technologies enable the analysis of complex biological large molecules, such as complete proteins. He reviewed some main areas of application of MS to proteins, including the identification of each peak of a protein digest, using the latest MALDI (matrix-assisted laser-desorption ionisation) TOF-MS, strand comparisons and “*de novo*” sequencing of hitherto unknown structures.

The use of these analytical systems, both independently and in combination, also provide an opportunity to produce a meaningful specification much more rapidly than before — but it is essential to tailor the derived specification to the use of the product. Process control and monitoring successive production stages (synthesis, polymorph resolution, solution mixing, blending, granulation, drying and coating) can also be addressed through scanning electron microscopy, solid phase NMR or reflective NIR.

Professor Dawson recognised current trends to ensure that “regulatory frameworks should reflect specifications that are case-specific” and considered that the huge analytical advances are now sufficiently robust for acceptance in drug application dossiers. In some instances, such as NIR, the techniques are “readily transferable to the third world” and, *inter alia*, useful in sorting at source adulteration of herbal products, at least qualitatively. He concluded that regulators should be “ready to accept new technologies earlier rather than later”.

ARRHYTHMIA TREATMENT

Discussing the mechanism of arrhythmia, Professor Luc Hondeghem (pharmaceutical consultant, Belgium) sought to resolve a paradox concerning the value of prolongation of the Q-T interval (the time between consecutive diastolic beats). He distinguished between prolongation of APD (action potential duration), widely described as a major mechanism for anti-arrhythmia, and Q-T prolongation which is frequently regarded as a surrogate endpoint for pro-arrhythmia. He differentiated three types of APD prolongation: reverse use dependence



Professor Bill Dawson: complex analytical equipment has become user-friendly

(R), beat-to-beat instability (I) and quasi-triangular fast polarisation (T). When over 700 chemicals were subjected to electrophysiological characterisation, it was found that prolongation of APD was associated with pro-arrhythmia. However, even for those chemical agents that exhibited R/I/T activity, prolongation of APD correlated directly with reduction of pro-arrhythmia.

He reported animal studies with sub-endocardial and epicardial recording of electrode potential differences: for statistical reasons, these tests required large numbers of animal hearts. He instanced increasing the concentration of almokalant in the rabbit heart, which led to increasing prolongation of APD and pro-arrhythmia. However, prolongation of APD with low concentrations of erythromycin that lengthened the APD without causing any R/I/T, actually reduced the pro-arrhythmia of almokalant while lengthening the APD. More generally, when agents markedly prolonged the APD without any R/I/T action, arrhythmias were reduced.

Professor Hondeghem described a blinded study, in which three pharmaceutical companies submitted 41 vials containing therapeutic agents to a SCREENIT computerised system, which automatically quantifies the APD prolongation and the R/I/T parameters in Langendorff perfused rabbit hearts. He reported successful identification of all 21 vials containing a drug known to prolong the Q-T, and all agents reported to be torsadogenic [potentially fatal “torsade”] in the clinic were also identified by the test as potentially dangerous. He summarised that the SCREENIT system had proved to be highly reproducible; it had correctly recognised APD prolongation, accurately identified pro-arrhythmic drugs, and all this without erroneously incriminating safe agents. This innovative technique that he had pioneered had involved far fewer experiments (in this case 20 instead of 154), a much shorter time to assess (one day instead of six months) and saved many animals from experimentation.

PHARMACOGENOMIC TOOLS

In the final paper, Dr Jeroen Aerssens (University of Maastricht, Netherlands) discussed means to “identify patients at risk of adverse drug reactions”. Asking “why do compounds fail during chemical drug development?”, he instanced variability in drug metabolism (in pharmacokinetic safety studies), difficulty in identifying patients who would respond to the drug (efficacy problem), or just plain unfavourable economics. How then could pharmacogenomics help, and would this revolutionise medical practice? Dr Aerssens offered “hope but also reality.” “Today, drug development is based on undifferentiated treatment of large populations” and one drug “does not fit all patients. . . . Tomorrow, however, drug development will take account of variation between individuals.”

He recalled that an individual’s genetic make-up largely determines his risk for adverse drug reactions and extent of drug effectiveness. He claimed that “advances in biotechnology and the human genome project have led to a new discipline of pharmacogenomics” and that this study aimed better to understand these inter-individual variations in drug responses. Dr Aerssens noted that variation between individuals means that a drug is often only effective in a sub-set of patients, depending on whether the relevant enzyme is metabolising “normally” or metabolism is by a mutant gene. He suggested that such genetic control might dramatically affect therapeutic ratios conventionally ranked into “toxic”, “effective” and “ineffective” dosage.

He had examined adverse drug reaction literature from 1997 to mid-2002. Out of 27 drugs frequently indicted in ADR studies, almost 60 per cent were metabolised by at least one enzyme with known functional (potentially genetic) variation. He commented that genetic variation affects toxicity, efficacy and dosing. Taking one specific example, he examined various abnormalities for atypical patients in respect of the enzyme TPMT (thiopurine methyl transferase), which is involved in S-methylation of a series of purine-analogue drugs, such as azathioprine.

He said that applications of pharmacogenomics relied on two main technologies: (i) identification and screening for inherited variations in genotyping and (ii) monitoring the expression level of thousands of genes by suitable microarray technology. He commented that these tools could be applied to identify patients at risk for ADRs during drug development. Taking a wide range of drugs, as different as cardiovascular and psychoactive agents, he reviewed their metabolism by one genotype, remarking that such knowledge was available in clinical research but rarely in general practice. Dr Aerssens concluded with a list of factors to consider in applying pharmacogenomics to ADRs: severity, frequency and homogeneity, balance between cost of drug and cost of ADR treatment, and consequences of false positive or negative diagnoses.—Contributed by Professor Geoffrey Phillips, a former secretary of FIP Laboratories and Medicines Control Services section.

The importance of standardisation techniques for herbal medicines

Standardisation techniques for herbal medicines was the theme of a symposium jointly organised by two FIP sections — the Medicinal and Aromatic Plants section and the Laboratories and Medicines Control Services section — on 4 September. Co-chairmen Professor Peter Houghton, King's College, London, and Dr Frans van der Vaart, Royal Netherlands Medicines Laboratory, stressed the importance of harmonised and validated methodology and unambiguous labelling for herbal medicinal products (HMPs).

OFFICIALLY RECOGNISED STANDARDS

Dr Keith Helliwell, William Ransom, UK, referred to the categorisation of three types of extract according to correlation with their clinical activity. These are (1) herbs such as senna, where the characterised constituents of HMPs are solely responsible for the therapeutic and clinical effects with a dose related response to quantified constituents, (2) herbs such as St John's wort, where the characterised constituents are not solely responsible for the therapeutic and clinical effects, and (3) those other herbal materials, such as valerian, where there were no characterised constituents responsible for the therapeutic and clinical effects.

He differentiated therapeutically active herbal materials, with examples, within five chemobotanical families: anthraquinone-based (eg, senna), a few solanaceous alkaloids, certain other alkaloids (such as cinchona and opium), tannin-containing plants, and a miscellaneous category that included digitalis and capsicum. For quantification, Dr Helliwell exemplified three alternative methodologies used in European Pharmacopoeia monographs: solvent/solvent extraction followed either by titration [as for *Belladonna*] or by spectrophotometry [cascara, cinchona] or using liquid chromatography [capsicum, opium]. He proposed five "ideals" for standardisation:

1. The quantification method should be applicable equally to initial herbal materials, to the primary extraction product and to the final dosage form
2. Methodology as simple as possible to achieve the required conditions
3. Realistic levels and limits of quantification
4. Methods to be stability-indicating
5. The results of the quantification should reflect the therapeutic effect

He illustrated the applicability of his five ideals to plant sources, and extract and dosage form, of four typical families of herbal material. For *ipecacuanha*, the solvent method for the various herbal starting materials differs from PhEur and British Pharmacopoeia monographs for the liquid

extract and the tincture, and from that used by industry for the final dosage form. The method was suited to realistic levels, and was stability indicating, but the reproducible quantitation does not necessarily match therapeutic activity because alkaloids are measured in total and there could be a significant difference from batch to batch in the ratio of the two main alkaloids, emetine and cephaeline.

For cascara, the official assay, which comprises extraction/hydrolysis/extraction followed by colour development and spectrophotometric measurement, is tedious and needs careful training; however, it eliminates most congeners and there is a reasonable correlation with therapeutic activity.

He instanced three distinct approaches to the assay of capsicum fruit: (i) total pungent constituents (capsaicin, dihydrocapsaicin and nordihydrocapsaicin), (ii) total capsaicinoids by UV spectrophotometry (*cf* British Pharmaceutical Codex), and (iii) individual capsaicinoids by HPLC, as in the PhEur monograph on capsicum. These methods were reasonably stability-indicating and correlated with therapeutic effect. Explaining that capsicum fruit normally contains less than 5 per cent nonanoyl vanillylamide, he said dosage forms with much larger amounts (up to 50 per cent) of this synthetic capsaicin point to adulteration.

His fourth example was opium, which reflected a "a saga of more than 30 years". Historically, there has been diversity of assay methods for different dosage forms. Since 1993, the classic PhEur/BP gravimetric assay for medicinal opium has been superseded by HPLC, whereas for various formulations (opium tincture BP, camphorated tincture BP and squill linctus opiate BP [Gee's linctus]) that assay has been replaced by the Radulescu (nitrite/ammonia) reaction. The current PhEur method is generally applicable to all forms, has simple extraction and chromatography, reflects realistic limits of quantification, is stability indicating and provides a reasonable correlation with the therapeutic effect.

In conclusion, Dr Helliwell emphasised that:

1. Herbal materials have been used for centuries for their therapeutic efficacy
1. Accurately controlled dosage is important to prevent discomfort or even death
1. Many current methods of standardisation were developed 50 or more years ago, and in many cases they are still the most applicable
1. Modern chromatographic methods are not suitable for herbal materials where the therapeutic activity derives from a complex mixture of many closely related compounds, which could still be quantified by traditional methods

HERBAL EXTRACTS IN THE EUROPEAN PHARMACOPOEIA

Professor Gerhard Franz, University of Regensburg, Germany, and chairman of PhEur group 13B, said that "perhaps 75 per cent of the HMPs in Western Europe contain extracts, mainly in the dry form", but this "industrial reality is not completely reflected in the PhEur, which contains more than 130 monographs on herbal drugs but only eight examples of quality defined extracts and tinctures". As a consequence, the general monograph on extracts and tinctures was recently revised as a basic framework monograph and as a future guideline for the establishment of individual extract- and tincture monographs.

There is usually a lack information on active pharmaceutical ingredient (API) and relatively few pharmacokinetic studies but there are compendial controls on characteristic marker substances, inert materials, allergens and toxins, cellulose and lignin. Dr Franz recalled that problems largely flowed from the huge variety of HMPs on the European market, containing many different types of extracts, produced by several methods, and using a broad spectrum of solvent systems. Generally, the production of different types of extracts is outlined and specified according to the obvious needs in industrial practice, eg, great variation in batches of ginkgo, but it is possible to consolidate in an average standardised product. The PhEur "Production statement" concept controlled a "dry extraction ratio" (DER) reflecting polarity, concentration and volume of solvent, manufacturing process, time, pressure and temperature. An example is chamomile maceration, where choice of solvent varies the API found. Such "statements" would also relate to extraction water quality (eg, potable water for extraction).

In conclusion, Professor Franz observed that the increasing development of new and more effective synthetic drugs has not been reflected in HMPs; nevertheless HMPs are being better defined, and incorporation of similar concepts of safety, quality and efficacy are included in monographs acceptable to regulators.

ABSENCE OF KNOWN ACTIVE COMPONENTS

Dr Ezio Bombardelli, president of Indena Spa, Italy, dealt with the controversial situation of acceptable standardisation of herbals with no known active components. He agreed that the main problem has been the enormous variety of plant products in several countries. He listed the top ranked 47 herbs in tonnage quantities in France, and in Germany, where more than 200 plants are used. Relatively few extracts have

modern documentation in terms of safety, efficacy and chemical standardisation comparable with the registration requirements for any chemical drug. For plants with no known active components, the plant material must be identified and properly characterised, with well chosen markers belonging to at least two different classes of chemical substances, and analytical methods thoroughly validated with respect to a defined component mixture. He proposed that instrumental methods, for instance, nuclear magnetic resonance and Fourier transform-infrared spectrometry, should be used to provide a generally recognisable fingerprint.

The ratio between originating plant material and extract could be hugely variable. Moreover, for HMPs in popular demand, there are limited natural sources world-wide, sometimes with ecological restriction on harvesting, and time is needed for agronomic development to large-scale cultivation. He gave several examples of variable composition found in practice: there is a wide range of fatty acids and alcohols in extracts from *Serena repens* (used for benign prostatic hyperplasia); there is variable concentration of caffeic acids and amides in *Echinacea* spp; and *Hypericum perforatum* extracts include many inactive compounds, such as pseudohypericin and flavanoids, with range of hypericin wider in "wild" crops (0.053–0.3 per cent) but around 0.15 per cent in cultivated varieties from four countries. He concluded: "A perfect standardisation is essential to obtain biologically reproducible data in terms of safety and efficacy and the preparation must be consistent over time, stable and be devoid of unpredictable toxicity due to degradation or poor quality, or selection, of the plant material."

QUALITY CONTROL OF ISOFLAVONES IN SOYA

Professor Maria da Graça Campos, University of Coimbra, Portugal, referred to the current interest in the role of isoflavonoids as phyto-oestrogens, particularly soya products also present in infant formula products. These are of interest in hormone replacement therapy in women and for treatment of prostatic cancer in men. Genistein is less effective for hot flush relief but no side effects have been reported other than allergic response in asthma patients to dust from extract of soya protein.

She emphasised the need for rapid, sensitive and precise assays to analyse the compounds involved in pharmaceutical formulations, mostly tablets, with a minimum of manipulation, and to compare the results with those from extracts prepared from soya seeds. She advised that processing of the extracts may alter the distribution of the forms and can result in the loss of some isoflavones through leaching and through removal of undesirable fractions. To avoid this problem, the isoflavones were analysed using reversed-phase HPLC, with diode array detection, under gradient conditions, and without prior separation from the complementary bioactive compounds present in

the tablets. She had confirmed by liquid chromatography/mass spectrometry that those bioactive impurities were eliminated in the first step of the HPLC gradient without producing any interference in the analysis. Coloured components were removed with a C18 pre-column and the isoflavone markers were stable.

The results that her group obtained with a series of tablets available on the Portuguese market suggest that there is no well-established dose/activity relationship, because the amount of isoflavones recommended is different for each product, with complex HPLC profiles exhibited by different herb species. She proposed to follow up this work with a more detailed project, involving wider collaboration — including the Lisbon national laboratory — with the objective of studying isoflavonoids obtained from soya seeds from different botanical sources, and also assessing these compounds for their pharmacological and toxicological effects in laboratory rats. Chemical studies would be performed to analyse isoflavonoid compounds from tablets and extracts from soya seeds. In parallel, pharmacological and toxicological studies, using rats, would evaluate the effects on oestrogen receptors, and the toxicity effects of these compounds in relevant tissues.

PREPARATIONS ON THE BELGIAN MARKET

Dr Jozef Corthout, Belgian Medicines Control Laboratory, Brussels, described some problems they had encountered in evaluating hypericum. His laboratory undertook post-market quality control of branded drugs sold in public pharmacies, including herbal drug preparations. A general problem was the different ways that a plant could be used — harvested from a different part of the plant, or as crude drug, or different kind of extracts, not necessarily in a standardised preparation or normalised to a certain quantity of active substances or markers. A further problem was that the product could be standardised with respect to a single constituent (active or not), or to a group of metabolites — for example, hydroxyanthracene glycosides, or total cascarosides, or specifically cascaroside A.

He preferred a colorimetric assay for analysing a group of related compounds, whereas the determination of a single constituent required the separative power of a chromatographic method such as TLC, GC or HPLC, with various options for detectors. Their analytical method was then validated for linearity over a 70–130 per cent range, for precision as repeatability of 18 replicates; and for accuracy by determination of recovery of a spiked analyte with 70, 100 and 130 per cent of the nominal amount. There were also problems with labelling as to content, the form used (such as salt, ester or ether), whether they were using a drug or a crude extract, and whether it was "normalised" to a fixed content.

Dr Corthout then discussed specific problems encountered when examining

eight preparations of St John's wort (two tablets, three coated tablets and three capsules), all claiming a certain content of hypericins. Using PhEur definitions, and standard methods where available, his group verified a series of parameters and confirmed compliance with uniformity of mass, identification and microbial quality for all the products. However, for one capsule preparation, the disintegration test did not comply with the PhEur and the total hypericin content varied according to assay method — 13–85 per cent of the label claim by HPLC or 27–176 per cent by colorimetry. He commented that these results showed the importance of a detailed and accurate label description combined with a defined assay method.

Dr Corthout concluded that quality control of HMPs is much more complex than for synthetic substance analysis; it is often uncertain what extract has been used in the preparation, or which assay has been relied upon. Label claims can be misleading.—Contributed by Professor Geoffrey Phillips, a former secretary of FIP section for Laboratories & Medicines Control Services.

IPSF

The International Pharmaceutical Students Federation was established in 1949, following an initiative by the British Pharmaceutical Students Association.

It is a non-political, non-religious organisation represented in more than 45 countries. It has 33 national pharmacy student associations as full members, plus a number of local student organisations as associate members. Individual membership is available to students, new pharmacy graduates and pharmacists who have been registered for less than five years.

The focal point of IPSF activities is its 10-day annual congress. This includes general assemblies, at which policy issues and future projects are discussed, plus symposia, workshops, a poster exhibition and social activities. Jointly with the International Pharmaceutical Federation, the IPSF also presents a students' day during the annual FIP congress.

IPSF projects include work on national and international educational and health issues and "village concept" schemes, in which pharmacy students work with others to improve the standard of living and health conditions in remote areas of developing countries.

A student exchange scheme gives IPSF members the opportunity to work in a branch of pharmacy in another country for a short period. The federation's publishing activities include project reports and a thrice-yearly news.

Those wishing to support IPSF through individual membership should apply to the IPSF Secretariat, International Pharmaceutical Federation, Andries Bickerweg 5, 2517 JP Den Haag, The Netherlands (tel +31 70 3 63 1925; fax +31 70 3 65 9047; e-mail ipsf@fip.nl; website www.ipsf.org).