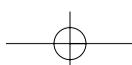


The Academy of Medical Sciences | FORUM

Safer Medicines Report

Safety Pharmacology working group report

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Summary of key findings and recommendation

A review of human safety information concluded the first administration to man of NCEs is very safe.

This suggests that the current paradigm for safety testing for untoward effects related to the major life sustaining physiological systems (respiratory, cardiovascular and major CNS effects) successfully prevents compounds being administered to man at concentrations that may cause these effects. The establishment of dose response in these models clearly fits with the successful identification of hazard and careful risk assessment prior to early clinical evaluation.

Ninety-five medicines withdrawn from the US market between 1960 and 1999 were reviewed to try to understand the main causes for ADR-related drug withdrawals. The principal interesting clusters of reasons were: arrhythmias – 12%; neuropsychiatric effects / abuse liability / dependency – 12%; hepatotoxicity – 9%; bone marrow toxicity- 7%; allergy- 6%.

Understanding proarrhythmia:

Considerable emphasis has been placed on studying drug effects on the QT interval. However, the real risk to human safety is the risk of causing Torsade de Pointes (TdeP), for which QT prolongation is a biomarker. The relationship between QT prolongation and TdeP is poorly defined and should be the subject of further non-clinical and clinical research. Such studies may support the safe development of novel therapies that may otherwise be discarded during non-clinical testing.

Abuse potential

The potential for centrally active drugs to have abuse potential and drug withdrawal reactions is an area of increasing regulatory interest. Although non-human primates are frequently used for these studies, literature data suggest that the rat has similar predictive value to man. A systematic comparison between the rat and non-human primate is required to identify the preferred species to predict this liability in man.

CNS pharmacology

Neuropsychiatric adverse events (such as somnolence, dizziness and asthenia) have been shown to be dose-limiting in a number of Phase I/II clinical trials as well as accounting for 12% of drug withdrawals from the market. In addition to these effects there is increasing concern related to the potential for CNS active drugs to cause adverse affective disorders (anxiogenic effects and suicidal tendencies). Animal models are used to detect drugs with the potential to have anxiolytic and antidepressant activity, although the value of these models to detect untoward affective effects is unknown. This should be an area of further research to develop animal models that can be used to test for anxiogenic/depressant activity.

Sensory function

The incidence of adverse drug reactions on vision and hearing is relatively low compared with the incidence of headache, nausea & vomiting, diarrhoea and dizziness, although the consequence to volunteers and patients may be greater. However, it is probable that symptoms (changes in vision and tinnitus) would be detected prior to the risk of long-term damage. Nevertheless, investments should be made to increase the understanding between drug effects on visual function in animals and man to further refine non-clinical testing paradigms. Furthermore, studies should be undertaken to develop predictive *in vitro* tests of ototoxicity.

Immune mediated adverse events of low frequency

There is confidence that currently available non-clinical models are able to detect immunosuppression and impaired host resistance despite the validation of these methods being incomplete. Methods to identify allergenicity have been widely used and for contact sensitization several approaches are validated. There remains a need to gain full acceptance for the methods available to detect respiratory and gastrointestinal sensitisers. Approaches for the identification of autoimmune responses or idiosyncratic immune

response have proven to be elusive and there is a need for more intensive study. This should include consideration of genetic susceptibility factors and environmental factors (diet, overall health status, life style etc). Furthermore, it is pertinent to ensure that all information relevant to a consideration of immune function from conventional toxicity studies, and an appreciation of cytokine expression patterns are included in an overall evaluation of impacts on immune status.

Training and education for safety pharmacology and clinical pharmacology

Formal training in life sciences within the UK has been steadily declining over the past 10 years and in particular under graduate and post graduate training involving the use of animals, in particular *in vivo*, in research has seen a marked decline. A clear need to invest in formal training within the UK exists. The number of pharmacology departments providing undergraduate training must not be allowed to reduce further. Post-graduate training needs to be supported more consistently with funds available to be targeted specifically at pharmacology and toxicology thus allowing development of safety pharmacologists with the appropriate experience. Initiatives within industry together with the BPS and BTS are welcomed but are insufficient to meet the future needs for industry, academia and often overlooked the regulatory bodies who will face dossier submissions containing data with increased complexity. Continual professional development and a formal accreditation system to support the development and maintenance of individuals involved in the non-clinical risk assessment of new medicines should be considered.

First in human paradigms

Early and more efficient evaluation of new molecules together with more rapid validation of novel targets **in humans** will lead to a major advance in the

identification of more efficacious medicines. The current requirements for non-clinical studies prior to first dose in man needs to be re-evaluated with the objective of permitting limited, highly controlled human studies to be conducted safely. Experience with Safety Pharmacology studies together with conventional toxicology studies has given reassurance that highly toxic compounds are readily detected. Furthermore, safety pharmacology studies have great potential to support first in human studies using 'novel' non-clinical safety paradigms.

Improving non-clinical testing paradigms through data sharing

Developing better non-clinical testing paradigms is in the interest of the patients, doctors, regulatory authorities and the pharmaceutical industry. Experience with groups such as ILSI/HESI has shown that considerable advancements in our knowledge of the predictivity of non-clinical tests can be made through the sharing of anonymous data. Such activities can effectively review current test paradigms, make recommendations for change and then assess the value of these changes stimulating a cycle of positive change.

In order to achieve the above proposals we recommend the creation of a UK centre, perhaps virtual, for development of Drug Safety Sciences. Such a centre would facilitate the sharing of data without compromise to commercial interest and enable structured assessment of the concordance between safety pharmacology studies in animal models against clinical outcome to be prospectively assessed and improved. Such a centre would also provide the academic focus to support the training of non-clinical and clinical pharmacologists and toxicologists.

Chapter one - Introduction

1.1 Studies conducted to investigate the pharmacology of new chemical entities (NCEs) are frequently classified as:

- primary pharmacodynamics (studies to investigate the designed mode of action expected to provide the desired clinical benefit);
- secondary pharmacodynamics (studies designed to explore the broader pharmacology of a compound e.g. actions not expected from its primary mode of action that may arise from additional actions of the compound);
- safety pharmacology (studies designed to investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposures within the therapeutic range and above).

1.2 Adverse events are defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. Adverse Drug reactions are a subset of Adverse Events that are thought to be causally related to the use of a medicine. Adverse Events and Adverse Drug Reactions can further be defined as seriousness criteria or by severity ratings (such as severe, moderate or mild). Serious Adverse Events are those that result in death, require hospitalisation or extension of hospital stay, are life-threatening, result in persistent or significant disability or are a cause of congenital abnormality. In general, severe adverse events prevent regular activities and are not relieved with symptomatic treatment. Moderate ADR's are bothersome, interfere with activities and are only partially relieved with symptomatic treatment with mild ADR's being slightly bothersome and are relieved with symptomatic treatment.

1.3 Non-clinical Safety Pharmacology testing (in combination with other non-clinical sciences) is directed towards prevention of serious ADR's in early clinical testing. This is approached by

defining the concentration/dose-response relationship for effects in non-clinical models that are used to predict the likely pharmacologically mediated adverse effects associated with either the pharmacology of the primary therapeutic target or with a secondary target that happens to be a property of the drug. Information from safety pharmacology studies is used to guide the starting dose and set likely stopping criteria in initial clinical studies and also provides guidance on potential adverse events for which monitoring in further clinical trials is appropriate. Safety Pharmacology also plays a role in understanding drug toxicities identified in humans during clinical evaluation.

1.4 The design and conduct of non-clinical safety pharmacology studies has been defined in ICH S7A (ICH S7A Safety Pharmacology Studies for Human Pharmaceuticals, CPMP/ICH/539/00) that was introduced in 2001. Thus, prior to the first administration to humans, core battery (cardiovascular, central nervous and respiratory system assessment) studies will be completed to investigate the safety pharmacology of the NCE. A further non-clinical guidance document on the conduct of Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarisation (QT Interval Prolongation) by Human Pharmaceuticals (ICH S7B) has also recently been finalised. Understanding the safety pharmacology associated with a new disease target has significant potential to reduce failure in development. Furthermore the design of Safety Pharmacology packages should be influenced by an understanding of the target, its distribution and function and not simply through fulfillment of ICH S7A. Mechanistic understanding will lead to better understanding of risk/benefit in man.

1.5 A literature review (Sibille *et al.*, 1998) and an analysis of Phase I clinical trials from 4 major pharmaceutical companies (the working group was blinded to the therapeutic class under evaluation) has demonstrated that the FIH trials are very safe with few, if any, serious ADRs being reported. This suggests that safety testing for

untoward effects related to the major life sustaining physiological systems (respiration, cardiovascular and major CNS effects e.g. convulsions) are successfully preventing compounds being administered to humans at doses that may cause these effects. It should also be noted that these initial clinical studies are conducted extremely diligently with intensive monitoring for the emergence of potential ADR's. Such careful entry into man has prevented serious ADR's occurring frequently even in drug classes commonly associated with high toxicity e.g. cytotoxic anticancer agents. Adverse events do occur in these early studies but are generally more frequently related to the procedure rather than the drug under study.

- 1.6** The above analysis from 4 pharmaceutical companies revealed with remarkable consistency that common ADRs (10–30% of volunteers) were: headache, nausea & vomiting, diarrhoea and dizziness. A large number of other lower frequency ADRs was reported – these tended to be specific to the molecule under investigation and often related to the mode of action. A review of the dose-limiting toxicities for 25 randomly selected molecules tested in single and multiple doses in healthy volunteers revealed that seven molecules had no dose-limiting ADRs. Interestingly, nausea and vomiting, diarrhoea and neuropsychiatric effects were dose-limiting in four cases but these ADR's were not readily identified from the non-clinical safety pharmacology data. Conversely, safety pharmacology testing clearly predicted effects upon the QT interval of the electrocardiogram, blood pressure effects, respiratory effects and diuresis. These findings are in agreement with the published literature that single dose non-clinical safety studies both under and over predict the effects seen in man (Olson *et al* 2000; Greaves *et al* 2004). Effects that frequently emerge during Phase 1 clinical evaluation and appear to be poorly predicted or extrapolated from the animal studies and warrant further development of predictive tests appear to be in the areas of GI disturbance and neuropsychiatric effects.
- 1.7** Approximately one in six drugs in clinical development is discontinued for clinical safety

reasons (Centre for Medicines Research 2003). Non-serious ADR's are often mechanism, drug class or disease related and may limit the utility of the medicine but usually do not pose a significant safety issue. Serious ADR's in Phase 2b or Phase 3 tend to occur at low frequency and may be related to the pharmacology or to the chemical properties of the drug. The former should be predictable from safety pharmacology assessment whereas the latter may induce toxicity via direct chemical toxicity or via hypersensitivity or immunological mechanisms. The impact of such toxicity is always very high requiring warnings, precautions and contraindications or even withdrawal from development or if detected post approval drug withdrawal from marketing.

- 1.8** The actual incidence of serious ADR's produced by marketed medicines is difficult to estimate accurately. ADR's are thought to be responsible for 4–6% of hospital admissions (Stephens 2004) with up to 106,000 deaths per year in the USA attributed to ADR's (Lazaraou *et al* 1998). The latter figure may be an over estimate but there is no doubt that ADR's cause significant patient harm and are a significant burden to health care. Although the frequency may be very low the establishment of the connection between pharmacological mechanism and the event may yield opportunities to reduce risk. For example the relationship between hERG potassium channel blockade, prolongation of the QT interval and induction of Torsade de Pointes (TdeP) has led to the rapid development of non-clinical screening tests that will help identify molecules with a reduced risk for this ADR.
- 1.9** Ninety-five medicines withdrawn from the US market were reviewed to try to understand the main causes for ADR-related drug withdrawals. The principal interesting clusters of reasons were: arrhythmias – 12%; neuropsychiatric effects / abuse liability / dependency – 12%; hepatotoxicity – 9%; bone marrow toxicity- 7 %; allergy- 6% (Stephens 2004). An earlier review of 121 medicines withdrawn from the worldwide market between 1960 and 1999 showed a similar spectrum with hepatic toxicity 26%, haematological 10%, cardiovascular 9%, skin effects 6% and carcinogenicity issues 6%.

- 1.10** With the above background it is clear that non-clinical safety assessment and safety pharmacology in particular is providing information that is preventing harmful candidate drugs entering clinical development, is identifying some key and important adverse events but is clearly failing to predict all adverse events. In considering areas for future research it is clear that much effort has been devoted to understanding drug induced QT interval prolongation. There clearly remains a need to understand drug induced pro-arrhythmia and its true relationship to altered repolarisation. Hepatotoxicity, bone marrow toxicity and immune mediated toxicity and GI toxicity are areas warranting further development. It is striking that neuropsychiatric effects account for a large group of drug withdrawals and they are also prominent as dose limiting toxicities in early volunteer patient studies that are not well predicted from the non-clinical models.
- 1.11** The study design and choice of species to be used in safety pharmacology studies needs to take into account a number of factors. Firstly, the known pharmacology of the compound, is the target under investigation expressed in the non-clinical species and/or does the compound interact with the animal target? This is of particular importance when studying the potential safety implications of the primary

pharmacology. Is the compound bioavailable and tolerated by the species under consideration? This may influence the choice of route of administration and therefore, the use of either conscious or anaesthetised animals.

- 1.12** Advances in technology and in particular the miniaturisation of telemetric devices will give opportunities to improve the non-clinical models and potentially positively influence 3R initiatives. There is a need to agree international acceptance criteria for the validation of new or improved non-clinical models. In addition, with the continuous recording of physiological parameters and concomitant measurements of drug plasma levels, these new technologies have great potential to be used to develop a better understanding of the pharmacokinetic: pharmacodynamic relationship in safety pharmacology studies. Such an approach may better inform clinical colleagues of the likely adverse effect concentration in man and potentially improve our confidence in the safety margins defined in non-clinical studies.
- 1.13** The changing demographics of the population with increasing numbers of elderly people requiring multiple-drug therapy is increasing the concerns around polypharmacy. There is a need to develop better testing paradigms.

Chapter two - General/secondary pharmacology studies

2.1 Actions of a potential drug at molecular targets (receptors, ion channel and enzymes) other than the desired therapeutic target may translate into deleterious, beneficial or no functional effects. Increased knowledge of the 'secondary' target interactions can be expected to lead to a better understanding of potential adverse pharmacology.

Current situation

2.2 Secondary pharmacodynamic studies have been defined as those that investigate the mode of action and/or effects of a substance not related to its therapeutic target (ICH S7A, 2000).

2.3 Secondary pharmacology has traditionally been assessed *in vivo* and effects observed followed up using a range of tissue preparations *in vitro*. Effects are quantified and a mechanism of action elucidated by application of *in vitro* studies which may be radio ligand binding assays, enzyme assays or cell/tissue based functional assays to determine which molecular target the drug has affinity for. The *in vivo* studies are performed where possible in conscious animals and involve investigation of the effects of the drug on physiological systems. This *in vivo* approach reveals unexpected effects in an integrated system, determines the therapeutic margin and so aids risk assessment when entering clinical development.

2.4 More recently the above approach has been reversed since it has become possible to screen for binding against a wide range of molecular targets (receptors, ion channels, transporters and enzymes), often derived from human tissue or human cell lines. The methods employed are predominantly radioligand binding or enzyme activity assays. For activity that occurs within the therapeutic concentration range additional studies to determine functional consequence of the secondary pharmacological activity is warranted. The methods used are cell/tissue assays together with specifically designed *in vivo* studies. This approach minimizes the use of

animals and facilitates the screening of larger numbers of compounds during candidate drug selection.

Challenges

2.5 The *in vivo* approach may not be sensitive to subtle effects and inter species variation needs to be considered when extrapolating from animal models to man. Should the animal target have higher affinity for the drug false positives will occur, potentially resulting in loss of valuable candidate drugs. These studies require the use of animals and so prompt ethical considerations;

2.6 The *in vitro* approach permits generation of large databases with defined 'hits' at specific targets. For many targets the correlation between binding affinity and functional consequence remains unclear. Often the target is a recombinant human target and an assumption is made that the affinity is identical to the native human target. A major question is whether *in vitro* radioligand binding assay data is predictive of secondary pharmacology effects in man. There is a clear need to establish confidence in such predictions;

Recommendations

2.7 Establishment of databases that bridge the non-clinical data with observations from clinical studies will clearly aid the predictivity of the non-clinical data. Access to data that summarise the non-clinical secondary pharmacological targets for marketed drugs would be the first step towards enhancement. Commercially available databases are being developed (DrugMatix™, BioPrint™ and Receptor selectivity mapping database are examples) but to fully exploit this knowledge access to data from compounds in development (including those that fail) as well as marketed drugs will greatly facilitate this understanding. Such a database could be established within a confidential framework should a centre for the safety assessment of drugs be set up.

2.8 The emergence of large databases and their considerable potential in this area highlight the need for bio-informatic expertise. There is clearly a need to focus training and development of this key skill. Furthermore, the availability of skills to integrate the molecular information with *in vivo* pharmacology has diminished. Focus on

developing infrastructure to produce highly skilled in-vivo pharmacologists is essential.

2.9 The development of databases that facilitate a better understanding of the functional consequence of secondary targets will aid development of structure activity tools leading to better drug design.

Chapter three - Cardiovascular and renal safety pharmacology

3.1 The evaluation of drug effects on the cardiovascular system can be effectively conducted using a range of techniques and non-clinical species.

Current situation

3.2 The development of telemetry techniques in conscious animals has had a major impact on the conduct of cardiovascular safety pharmacology studies. These techniques support the continuous collection of a range of physiological data, including heart rate, blood pressure, left ventricular pressure, ECG and body temperature over 24 hours. Thus, drug effects can be studied under 'normal' physiological conditions often using the clinical route of administration (e.g. oral or intravenous). The use of these technologies also support using a cross-over study design (each animal receives active treatment and vehicle) that provides a high statistical power to detect drug effects which, in turn, reduces the number of animals used in each study. In addition, using telemetered techniques drug effects can be studied at plasma C_{max} and C_{min} levels with the potential to define the pharmacokinetic: pharmacodynamic relationships. Furthermore, effects of metabolites may be investigated. Echocardiography applied to animal studies is a valuable technique that is probably under utilised. It is also important to consider the need to measure regional blood flow on a case-by-case basis.

3.3 Anaesthetised animals can be used when, for example, the compound is poorly tolerated in the chosen species (for example due to emesis) or relatively little is known about the compound at the time of evaluation. This raises the complication of the unknown effect of anesthesia on responses to the compound under study, although it is the view of this working group that, at least the anaesthetised dog responds to cardiovascular effects of drugs in a qualitatively similar extent as the conscious dog.

3.4 The concordance between cardiovascular effects of drugs in animals (in particular dogs and

primates) and in humans is good. This is consistent with the low incidence of cardiovascular ADRs in FIH trials and the use of similar animal models in Drug Discovery to detect novel cardiovascular products as new therapeutics.

3.5 Of great scientific, medical and regulatory interest is in better understanding adverse drug effects on cardiac repolarisation (QT interval of the ECG) and its associated risk of causing the life threatening arrhythmia, TdP. Several studies have established that a vast majority of compounds that prolong the QT interval in man do so *via* inhibition of the potassium current, I_{Kr}. Conversely, not all I_{Kr} blockers prolong the QT interval *in vivo*. During the last 2–5 years, significant advances have been made in our ability to test for drug effects on the I_{Kr} current and a range of other cardiac ion channels. These *in vitro* assays, in conjunction with the above *in vivo* cardiovascular/cardiac studies, are able to detect a vast majority of compounds known to prolong the QT interval in man. The validity of these assays has been established through ILSI/HESI and also JPMA initiatives. Both of these groups studied a range of compounds with known torsadogenic risks in man in comparison with non-torsadogenic compounds and were able to differentiate between these two classes of compounds.

3.6 Thus, the risk of drug-induced changes in cardiac repolarisation in man has been greatly reduced. Despite these advances a major area of challenge in the cardiac/cardiovascular field remains the understanding of the relationship between drug effects on cardiac repolarisation and the risk of causing cardiac arrhythmias. This is an area that this working group recommends further investment in both non-clinical and clinical research (see section 1).

3.7 The effects of NCEs on renal function can be studied in conscious animals, frequently the rat, following oral dosing. The primary end points are urine volume and electrolyte concentrations. Although the methods used have not changed greatly in the last decade or so, the low incidence of renal adverse effects would

suggest that they are effective at detecting risks for human safety.

Challenges and opportunities

- 3.8** Greater understanding of structure-activity relationships will enhance drug candidate selection. However, there is no crystal structure of the hERG channel known at present due to its membrane bound nature. Site directed mutation and homology models based on the template bacterial KcsA channel have been used to infer important amino acid residues likely to be involved in the drug/channel interaction. Such models may support *in silico* screening to minimise drug interactions with the hERG channel. Studies to investigate drug effects on cardiac ion channels should of course include other ion channels such as sodium and calcium. Furthermore, understanding pharmacogenetics and the importance of ‘outliers’ is likely to give significant benefit in assessing human risk of cardiovascular drug toxicity.
- 3.9** The potential to expand *in silico* models such as, “Cardioprism™”, using the IC₅₀ profile for different ion channels and a library of validated computer models to simulate the effects of compounds on *in vitro* preparations should be explored further. The library contains *in silico* models of the sinus node, Purkinje fibers, atrial and ventricular myocytes including epicardial, midmyocardial and endocardial cells may have future value to reduce adverse cardiac effects of new potential drugs. Furthermore, these models are customized to reflect different species common in non-clinical testing, namely rabbits, dogs, and guinea pigs.
- 3.10** The main area of challenge in the cardiovascular field is the improvement of the understanding of the relationship between drug effects on cardiac repolarisation (QT interval) and the risk of causing TdEP arrhythmias, since the former is only a biomarker of the latter. The characteristics of subpopulations such as diseased, young and old individuals as well as male/female differences have not sufficiently been investigated. TdEP arrhythmia is an area

that this working group recommends further investment in both non-clinical and clinical research.

- 3.11** The challenges for the future include the further development of telemetry methods to support the recording of more physiological end points in a single animal. Companies are developing methods to record pulmonary mechanics, so the ideal would be to study drug effects on cardiovascular, cardiac and pulmonary function in a single non-rodent animal model. This would provide an excellent evaluation of the main physiological systems of concern to the safety pharmacologist and reduce and refine the use of animals in non-clinical testing.
- 3.12** Consideration should be given to understanding drug effects under conditions of ‘physiological challenge’, for example, to improve our ability to detect drugs that may cause postural hypotension.
- 3.13** Changes in cardiovascular function can lead to pathological consequences in toxicology testing. It is therefore important, and a current challenge, to integrate findings in cardiovascular safety pharmacology studies with those observed in regulatory toxicology studies.

Proposals and recommendations

- 3.14** Considerable emphasis has been placed on studying drug effects on the QT interval. However, the real risk to human safety is the risk of causing TdEP, for which QT prolongation is a biomarker. The relationship between the magnitude of QT prolongation and TdEP is poorly defined and should be the subject of further non-clinical and clinical research. *In vitro* animal models are being investigated such as the rabbit Langendorff and ventricular wedge preparation to identify potential non-proarrhythmic QT prolonging agents. Such studies may support the safe development of novel therapies that may otherwise be discarded during non-clinical testing.

Chapter four - Respiratory Safety Pharmacology

Current situation

- 4.1** The ICH S7A guideline for safety pharmacology clearly defines a need for evaluation of potential effects upon the respiratory system prior to entry into man.
- 4.2** The respiratory system has approximately 60 different cell types involved in its homeostasis and so it is particularly complex to study and interpret drug-induced effects. It has been simpler to define effects upon the respiratory system as mediated through the structures involved with movement of air or through gas exchange. Broad classes of pharmacological responsiveness can be discriminated through the functional changes induced. For instance sensory irritants generally decrease respiratory rate whereas pulmonary irritants increase rate.
- 4.3** There are major challenges in extrapolating between species. For example aspirin induced asthma is common in humans with 21% of adults and 5% of children affected yet there is no predictive animal model. Evidence from cross sensitivity between other non-steroidal anti-inflammatory (93–100% of aspirin sensitive patients responding to ibuprofen naproxen and diclofenac) and paracetamol (2% cross reactivity) suggest clear mechanistic differences and possibly a genetic involvement. These observations taken together with the availability of over 25 years of published clinical findings with potentially fatal aspirin induced bronchoconstriction suggest effects upon the respiratory system are under reported.
- 4.4** The absence of frequent or profound adverse reaction in the respiratory system during initial clinical evaluation suggest non-clinical testing, as currently performed, appears to prevent major respiratory reactions in early clinical development. In a study reported by Sibille *et al* (1998) there were no life threatening events in 1015 healthy volunteers during 54 Phase 1 studies. However, the profound consequence of an adverse event (even if rare) is illustrated by the death of a volunteer following administration of

hexamethonium to the respiratory tract without adequate non-clinical evaluation by this route of administration. Adverse events in the respiratory system that led to withdrawal from the market are rare, accounting for only 2 of 121 if anaphylaxis is excluded.

- 4.5** The incidence and extent of adverse reactions in the respiratory system are probably poorly understood, particularly the influence of disease, pharmacogenetics and drug-drug interactions. Complications from agents affecting respiratory pattern during sleep is similarly poorly understood. The animal models available to assess respiratory function are complicated by clear species differences. For instance alveolar surface area shows marked difference between humans and rats leading to very different concentrations present in lung fluid following an equivalent inhaled dose. Experience with insoluble particulate compounds has shown the rodent is good at hazard identification but poor in defining risk because of the alveolar macrophage ‘overload’ phenomenon when this cell type is key for clearance.
- 4.6** Technology to measure lung mechanics, particularly in unrestrained conscious animals, is under-developed. The tests identified in ICH S7A regarding respiratory function monitoring define (Murphy, 2002) a basic minimum requirement but in some instances suggest inappropriate insensitive measures such as blood gas analysis.

Challenges and opportunities

- 4.7** The ability of non-clinical studies to detect chronic pulmonary effects is poorly understood, together with our ability to interpret the clinical concordance of the data. The ability to detect potential risks to human safety would be enhanced following future enhancements in respiratory technologies. Many features of the multi component respiratory system are best studied in the integrated whole animal. *In vitro* studies while useful to explain specific cellular target toxicities are unlikely to be helpful in assessing functional respiratory function. The

focus should therefore be on development and refinement of animal models of human respiratory function. Murphy (2002) has classified the respiratory system into two compartments, the ventilatory pump and the gas exchanger. Respiratory depression is accompanied by changes in ventilatory parameters (rate, tidal volume, air flow and airway resistance together with lung compliance as a measure of 'elasticity') which although technically demanding can be measured. Greater adoption of these methods in safety pharmacology will facilitate identification of drugs that adversely affect the respiratory system. It is clear that improved methods for detecting airway obstruction are needed.

- 4.8** The development of micro – sensors and transponders has led to opportunities in developing telemetric methods for use in unrestrained conscious animals. This will open opportunities to broaden the species used in respiratory function testing. Experience to date has focused upon the rodent with expertise in other species restricted to a few specialist centres. The validity of these new methods would benefit from a wider evaluation of drugs known to affect the respiratory system in man through comparison of the changes in FEV1 (routinely carried out in clinical evaluation of drugs known or suspected of adverse respiratory

effects) in man with changes in non-clinical parameters.

Proposals and recommendations

- 4.9** The low incidence of known severe respiratory ADR's suggests the current general approach should not be minimized.
- 4.10** The present regulatory focus within ICH S7A of using blood gas analysis techniques (or pulse oximetry) solely as a means of non-clinical respiratory safety assessment is inappropriate due to the insensitivity of these approaches. Hence, the content of ICH S7A is suboptimal and needs further modification to provide the best non-clinical datasets for regulatory submissions.
- 4.11** An increased willingness to share data for drugs not taken into human clinical development and those with clinical experience to fully understand the frequency and significance of changes in non-clinical models would greatly improve the understanding of their predictive value. This could be facilitated by creation of a centre dedicated to safety evaluation of medicines. The routine inclusion of simple respiratory function testing during early clinical evaluation should be considered.

Chapter five - Central nervous system (CNS) safety pharmacology

Current situation

- 5.1** Effects upon the CNS are a significant cause of adverse drug reactions ranging from non-compliance with drug therapy as a consequence of, for example, weight gain, nausea or anxiety to severe reactions such as convulsions. Less readily recognizable effects are also important as a consequence of an aging population resulting in a higher prevalence of neurodegenerative diseases. Effects mediated through an adverse reaction in the CNS have been considered under the following headings:

Abuse Potential and Addiction Liability

- 5.2** The potential of centrally active drugs to be associated with abuse potential/addiction liability is an area of increasing regulatory interest. Although studies to investigate these properties are not undertaken on a routine basis in safety pharmacology studies they should be considered as part of the investigation of the broader secondary pharmacology properties of a NCE. Although the non-human primate is studied extensively there is considerable evidence to support the use of the rat in self-administration studies to predict abuse potential in man.

Drug induced Dizziness

- 5.3** The causality of dizziness is often ascribed to drug treatment, despite the subjectivity of the diagnosis and the fact that it rarely causes drug withdrawal. There does not appear to be a good non-clinical model to predict this effect in humans. It is unlikely, given the difficulty in designing objective human measures, that a realistic animal model will be valuable and accepted since the mechanisms associated with dizziness are diverse.

Nausea and Vomiting

- 5.4** Nausea and vomiting are common adverse effects of drugs often mediated through the CNS

that affect drugs in all stages of development. It is estimated that nausea and vomiting occur in approximately 30% of initial drug trials and in approximately 10% of phase 1 studies this effect dose limiting. The non-clinical safety pharmacology studies did not reliably predict the effect seen in man suggesting better models are required.

Food intake, weight gain and weight loss.

- 5.5** Several classes of drugs have been associated with disturbance of food intake and/or bodyweight. The effects are generally of greater consequence in the very young, elderly or severely ill patients. These parameters are routinely measured in conventional toxicology studies. Furthermore, specific models to assess appetite are also available and appear to have good predictive utility. There does not appear to be a need for development of further more sophisticated tests other than to follow up specific drug related findings.

Neuropsychiatric disorders

- 5.6** Non-clinical evaluation of drugs that do not penetrate the blood brain barrier does not routinely include any sophisticated assessment for centrally mediated CNS effects such as anxiety, depression, mood disturbance, learning and memory or dependence. The current approach to assess specifically drug-induced changes is prompted by evidence of brain penetration or signals for the initial broad-spectrum assessments – Irwin or Functional Observational battery. In addition, animal models are widely used to detect primary pharmacological effects that may have utility in the treatment of anxiety and depression. However, the potential of these models to detect untoward drug effects (anxiogenic and depression) is poorly defined. Nevertheless, given the prevalence of neuropsychiatric disorders in both clinical development and in clinical use post marketing this approach should be re-considered.

Challenges and opportunities

- 5.7** Given the high incidence of nausea and vomiting observed in clinical trials either greater emphasis on observations in non-clinical safety studies is required or improved models to predict these effects in man are required. The ferret, shrew and marmoset show promise as useful species. This is an area where better prediction aiding design of new drugs may yield considerable human benefit.
- 5.8** The potential of animal models (currently used to detect agents that may be of benefit in the treatment of anxiety and depression) to identify potential anxiogenic and prodepressive effects is unknown and should be studied further, although it must be recognized that animal models used to detect, for example, antidepressant drugs are not animal models of depression.
- 5.9** The value of the rat as a model to detect potential abuse potential of CNS active drugs, in comparison to the non-human primate, should be thoroughly investigated.
- 5.10** The potential of new techniques to study CNS effects of NCEs should be considered. For example, the measurement of high-frequency ultrasonic vocalisations (USVs) in the rat. Ethological studies have revealed that rats make

USVs in many different social and emotional situations. Measurements of USV's in e.g. the open field assessment as part of the functional observational battery (FOB) may lead to the identification of untoward drug effects.

Proposals and recommendations

- 5.11** The potential for centrally active drugs to have abuse potential is an area of increasing regulatory interest. Although non-human primates are frequently used for these studies, literature data suggest that the rat has similar predictive value to man. A systematic comparison between the rat and non-human primate is required to identify the preferred species to predict this liability in man. In addition to these effects there is increasing concern related to the potential for CNS active drugs to cause adverse affective disorders (anxiogenic effects and suicidal tendencies), as well as abuse potential and drug withdrawal reactions. Animal models are used to detect drugs with the potential to have anxiolytic and antidepressant activity, although the value of these animals to detect untoward affective effects is unknown. This should be an area of further research to develop animal models that can be used to test for anxiogenic/depressant activity.

Chapter six - Hepatic Function

Current situation

- 6.1** Mechanisms of drug induced liver dysfunction are complex and remain relatively poorly defined and require better mechanistic understanding. Liver toxicity and functional disturbance is probably the most common serious adverse event seen in drug development and has account for 9% of drug withdrawals from the US market.
- 6.2** Drug induced liver dysfunction is detected frequently during both non-clinical and clinical evaluation and is the cause of significant attrition and project delay.
- 6.3** Despite non-clinical models readily identifying hepatotoxicity there are many examples where non-clinical models have failed to adequately identify or characterise the risk to man. Many of the drugs currently on the market are known to have the potential to cause adverse effects on liver function in certain susceptible individuals (Pritchard et al 2003, Olson et al 2000; Kaplowitz 2001; Boelsterli 2003; Lee 2003.). In some cases the incidence and severity warrants drug withdrawal (e.g. Troglitazone) in others dysfunction is mild and remains asymptomatic (eg Gentamycin).
- 6.4** Relatively few case examples of liver toxicity have been studied in depth and even where this has been undertaken our understanding remains incomplete e.g. paracetamol.
- 6.5** Liver toxicity that occurs with a very low incidence (idiosyncratic) is poorly understood and in particular the individual susceptibility factors are sparsely known. There is a lack of appropriate experimental models.
- 6.6** Greater understanding of the role of drug transporters in the function of the liver is emerging and will potentially give better insight to mechanism and predictability of hepatotoxicity. In particular the role of transporters in drug induced cholestatic injury and the abnormal expression of bile salt transporters in disease and drug-induced injury is emerging. Hereditary defects in individual bile salt transporters have been associated with familial intrahepatic cholestasis type 1 (FIC1 gene), type 2 (BSEP gene), type 3 (MDR3 gene) and Dubin Johnson Syndrome (MRP2 gene). It is notable that several drugs that cause liver dysfunction have been shown to impair the transport activity of BSEP (Stieger et al 2000).
- 6.7** Diagnosis of liver toxicity is problematic. This is linked, at least in part, to our incomplete understanding of underlying mechanisms. Some compounds cause pathological alterations that do not result in overt toxicity as assessed by evaluation of plasma levels of “liver enzymes” (e.g. biliary hyperplasia), while sporadic mild elevations of liver enzyme levels that are not accompanied by significant liver dysfunction occur frequently in the human population. The “gold standard” criteria for assessing toxicity to the liver in animals plus man are liver histology plus elevated plasma liver enzyme levels. Evaluation of liver histology has great value but can be difficult to assess in man because of ethical and safety issues. Also, evaluating small biopsy samples can be misleading – so diagnostic liver biopsies in man are less favoured now for diagnostic purposes than they were 20 years ago. ALT assesses hepatocellular damage but is not truly liver specific. Significant elevations in ALT exceeding small multiples of normal control range (with changes less than 1.5-fold generally considered not significant) are considered suggestive of liver damage. A combination of a 3-fold elevation in ALT plus 2-fold elevation of bilirubin is generally taken to indicate liver toxicity in man. Other plasma markers of liver toxicity that are widely used include γ -GT, AST, ALP (indicative of cholestatic damage) and bilirubin.
- 6.8** Distinction between toxicity and adaptive response by measurement of traditional clinical chemistry parameters is currently not achievable. Identification of better biomarkers, chemical or imaging for liver injury would greatly improve diagnostic capabilities.

Challenges and opportunities

- 6.9** There is a clear need for improved biomarkers for liver dysfunction. The current methods are widely used but old and in some circumstances unspecific. Opportunities to use improved imaging together with proteomic and metabonomic methods should yield new markers for evaluation.
- 6.10** Development of improved *in silico* and *in vitro* approaches will aid design of drugs with reduced hepatotoxicity potential. Structure-toxicity databases need to be defined – but before this is feasible we need an improved understanding of the role played by chemical structure as a determinant of drug-induced liver dysfunction. This should follow from a better understanding of mechanisms. The most promising avenues currently are prediction of formation of chemically reactive metabolites and transporter interactions. More complex *in vitro* 3-D models (e.g. liver spheroids) and liver slices, which have the advantage of more relevant cell architecture, are an attractive option but require further development.
- 6.11** Searching for genetic and environmental susceptibility factors that can pre-dispose certain patients to drug induced liver injury. Although some progress is now being made in identifying genetic factors, progress to date has been slow.
- 6.12** Improved *in vivo* models for assessing compound-induced liver dysfunction and for circumventing species variability. These should be linked to mechanistic considerations. Transgenic technology could give us “humanised” experimental animals, which improve the human relevance of non-clinical safety testing in animals. Perhaps generating mice that express humanised bile canalicular transporters (BSEP, MDR1, MRP2 etc.) could provide a relevant model system for improved assessment of hepatobiliary toxicity. In this regard, an improved understanding of ADME properties in animals vs. man should also be of great value.
- 6.13** Improved ability to define mechanisms of liver toxicity, once this is observed in man.

Appropriate use of the new approaches alongside conventional toxicity investigations, to define key molecular events and susceptibility factors offer the best opportunities to understand mechanism. The role of biliary efflux transporters in hepatobiliary toxicity is a particularly promising avenue to explore, as is the role played by reactive metabolites.

Proposals and recommendations

- 6.14** More consistent and accurate detection/diagnosis of drug induced liver dysfunction during clinical trials (rather than post-marketing) using existing technologies with conventional liver function tests supported by more sophisticated causality assessments can be expected to lead to better and earlier identification of liver toxicity. This should be complemented by research focused on more sophisticated application of existing approaches (most notably causality assessments), and on detection of novel biomarkers of liver dysfunction.
- 6.15** An improved ability to predict and avoid drug-induced liver dysfunction prior to introduction of drugs into man. Significant progress in this area is likely to require a more sophisticated understanding of the key molecular and cellular mechanisms that result in drug-induced liver dysfunction and that influence species differences in toxic responses. Particular attention should focus on the roles played by drug disposition, chemically reactive metabolites, and transporters interactions. The outcome from this work needs to be linked to development and validation of structure-toxicity databases, and of *in vitro* experimental approaches, that can be used by medicinal chemists and bioscientists to design and select safer compounds during drug discovery.
- 6.16** Identification of susceptibility factors that determine why some individuals are markedly more susceptible to drug-induced liver dysfunction than the general population. These susceptibility factors are likely to be multifactorial, and could well involve a complex interplay between genetics and environment.

Chapter seven - Gastrointestinal (GI) safety pharmacology

Current situation

- 7.1** Adverse reactions in the gastrointestinal tract appear to occur at all stages of drug development and in subsequent clinical use. Drug-induced disturbances of GI function negatively impact patient safety, compliance, quality of life and clinical benefit. The impact of GI ADRs in terms of drug withdrawals from the market has been minimal, with only one example, pirofen, in the period from 1960 to 1999, however, drugs with labeling restrictions are commonplace, for example Lotronex (Fung *et al.*, 2001).
- 7.2** GI ADRs are some of the most frequently reported in all phases of clinical drug development and for marketed products, as illustrated by the 700 drugs that are implicated in causing diarrhoea. Furthermore, GI ADRs account for approximately 18% of all reported clinical adverse drug reactions and 20 to 40% of those in hospitalised patient. Given, however, that symptoms of disturbed GI function are encountered in everyday life, the actual incidence of drug-related effects are most likely extensively under-reported. The majority of reported ADRs are functional in nature (nausea, vomiting, dyspepsia, abdominal cramps and diarrhoea or constipation) with a lesser number related to lesions (e.g., ulceration) or enhanced susceptibility to infection (e.g., pseudomembranous colitis). Of these, it is estimated that approximately 80% are Type A predictable pharmacological reactions. Some of the most numerous and serious GI ADRs are attributed to chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs). Widespread NSAID use and associated reports of gastric complications, has given rise to the acceptance that NSAID use carries a significant risk of developing peptic ulceration. In a recent prospective analysis of 18,820 patients in a UK hospital, 29.6% of all ADR cases were related to NSAID. Furthermore, NSAID use in the US alone is estimated to be responsible for over 100,000 hospitalisations and 1,700 deaths per year.
- 7.3** The challenge of defining the non-clinical GI liability of a potential new medicine is not only hampered by biological complexity of the system under scrutiny, but also by fundamental differences in terms of GI function between the rat, the most commonly used species in research, and man. For example the rat does not possess a gall bladder and does not vomit. This raises important questions about the relevant species for conducting GI assessment. Based on GI functional homology for man, especially motility, gastric emptying and pH, particularly in the fasted state which is analogous to the conditions prevailing in many Phase I trials, the dog, is perhaps a more relevant species. Moreover, the dog was a better predictor of clinical GI ADRs than the monkey for 25 anticancer drugs. Although physiological similarity is an important requirement it is only one of many factors that must be considered during species selection.
- 7.4** GI ADRs are some of those most frequently encountered in healthy volunteers and patients, yet assessment of potential new medicines on the GI system is not a regulatory requirement before conducting Phase I trials, nor even for marketing approval. It is, however, recommended in the ICH S7A Guideline for Safety Pharmacology Studies (CPMP/ICH/539/00) that the GI system should be studied on a case-by-case basis. The ICH guideline does not classify GI system as a “core” battery organ system, unlike the respiratory, central nervous system and cardiovascular systems, of which assessment is mandated prior to Phase I trials. Moreover, the GI system is relegated to a group of supplemental organ systems to be studied only where there is a cause for concern.
- 7.5** Further to the list of parameters in ICH S7A, a recent publication has reviewed GI models and techniques applicable for use in safety pharmacology. One important element omitted from both ICHS7A and the review by Harrison *et al.*, (2004) of particular relevance to anticancer drugs is nausea and vomiting. There is no doubt this omission reflects the

complexity of the symptom and that there are no non-clinical models able to relay nausea. There is, however, a growing understanding of the causes nausea and vomiting and non-clinical dog and ferret models amenable for safety pharmacology testing.

- 7.6** The influence of GI intolerance on patient compliance to medication is probably under reported.

Challenges and opportunities

- 7.7** A comprehensive review of non-clinical GI endpoints to determine the concordance between non-clinical GI safety models and man, to identify and promote appropriate models/species and relegate and exclude irrelevant misleading models/species is needed. Current methods to assess GI function despite being well established have poorly understood predictive value. A clear need exists to develop a better understanding of the models available and their applicability to drug evaluation.
- 7.8** Given its complexity the GI tract is a system probably best studied *in vivo*. Despite this complexity there are clear advantages in understanding molecular targets associated with GI dysfunction and this should be an area for future research. There are opportunities to combine key GI parameters (eg secretion and motility) into single *in vivo* studies. To facilitate understanding of GI risk in humans a better understanding of the importance of local

concentration rather than systemic concentration/response is needed.

- 7.9** Two common major dose limiting effects, nausea and emesis, are poorly understood and predicted. Better models are required.

Proposals and recommendations

- 7.10** Placing greater importance on GI-related observations (e.g., emesis) in acute and repeated dose toxicology studies, including seemingly isolated incidences may help trigger formal studies of GI function if not conducted routinely. The opportunities to use non-invasive imaging techniques may enhance the predictive capability of these tests and should be encouraged.
- 7.11** There is a clear need to develop and validate non-clinical methods for detecting nausea and emesis potential. Reducing these important side effects would be expected to facilitate drug development and also improve patient compliance for marketed drugs.
- 7.12** Encourage and nurture links between GI specialists in academia and industry. In particular there is a need to understand better the predictive value of the non-clinical models. Establishment of a Safety Sciences centre within the UK that can act as a repository of data would greatly help understanding of the existing data available within individual companies.

Chapter eight - Sensory Safety Pharmacology

Current situation

- 8.1** Adverse drug effects in man on the main sensory organs, sight, hearing, taste and smell are relatively poorly understood and occur with a relatively low frequency when compared with the incidence of headache, nausea & vomiting, diarrhoea and dizziness. However, untoward effects on the sensory organs, especially hearing and sight, could have a significant impact on volunteers/patients well being.
- 8.2** Behavioral pharmacology studies may detect untoward effects on balance and sight, although it is probably that significant deleterious effects on these systems would be required before changes in behavior would be detected. In toxicology studies a detailed investigation of drug effects on the eye are conducted as part of the clinical examination of animals is performed (corneal reflex (dog), anterior chamber and lens examination using a slit lamp and examination of the vitreous body and the retinal fundus using an indirect ophthalmoscope and a magnifying lens). In addition, histological examination of potential effects on the structure of the retina and optic tract are conducted.
- 8.3** Sophisticated non-clinical tests are available to investigate drug effects on the retina (the electroretinogram, ERG) and visual pathway (visual evoked potential, VEP). Pharmacological effects on the ERG have been reported with a range compounds with varying mechanisms of action. Such studies, when used in conjunction with histopathology in toxicology, can be used to establish a reversible pharmacological action on the retina that would be less of a concern with respect to human safety, or an irreversible toxic effect on the retina that would be of great concern for human safety. In the event of visual adverse events being reported in man, the ERG in both man and animals is a valuable investigative tool to establish the site of action of a compound and to provide a link between non-clinical and clinical data to build confidence in the long-term safety of a drug. The challenges for the future would be to further characterise the relationship between drug effects on visual function in man and in animals to established the predictive value of existing test systems and to develop novel assays.
- 8.4** Dizziness is a frequently reported adverse event in man. This may arise through changes in blood pressure (postural hypotension), an effect on the central nervous system (CNS) or an effect in the inner ear. Non-clinical models are available to investigate drug effects on balance and motor coordination, such as the rota-rod and activity box, although the sensitivity of these models to detect adverse drug effects on balance *via* an effect on the ear has not been systematically studied.
- 8.5** Ototoxicity is thought to be rare, but possibly under reported (essentially confined to loop diuretics, aminoglycosides, anti-neoplastic cytotoxics, aspirin and quinidine) although the impact on the individual if affected is significant. There is no safety pharmacology test used to determine potential effects of NCEs on hearing and there is no regulatory requirement to evaluate ototoxicity unless there is a clear cause for concern. However, early signs of ototoxicity (tinnitus) can be detected in volunteers and patients that would be a signal to stop therapy prior to irreversible damage. This is an area that requires basic research to systematically investigate the predictive value of non-clinical assays using a diverse range of pharmacological agents. Until such tests are in place, non-clinical testing for effects on hearing cannot be recommended.
- 8.6** Electrocochleography measures signals at the beginning of the vestibulocochlear nerve. An immediate reduction in action potential has been detected within minutes of an intravenous dose of aminoglycoside. Brain Stem Auditory Evoked Response (BAER) is a technique for measuring hearing loss. These techniques can be used in both humans and animals. However, this is an area that requires basic research to establish if predictive non-clinical assays can be established. Until such tests are in place, non-clinical testing for effects on hearing cannot be recommended.

Challenges and opportunities

- 8.7** As discussed above pharmacological effects on the ERG have been reported with a range of compounds with varying mechanisms of action. Results from non-clinical ERG studies are best interpreted in conjunction with histopathology. The challenges for the future is to further characterise the relationship between drug effects on visual function in man and in animals to establish the predictive value of existing test systems and to develop novel assays. Furthermore, a more comprehensive understanding of the relationship between histological changes to the visual pathway and changes to the ERG and VEP is required.
- 8.8** The ototoxicity potential of drugs is not routinely evaluated non-clinically which reflects the low incidence of ototoxicity in man and the fact that clinical signs of tinnitus can be detected in clinical trials. Furthermore, the predictive value using a diverse range of pharmacological agents of the existing animal models to man is unknown. The challenge, therefore, is to further our understanding of the concordance between effects of ototoxic drugs in animals and in man and to identify appropriate non-clinical testing strategies appropriate for the risk.

Proposals and recommendations

- 8.9** Although safety pharmacology methods (ERG and VEP) are available to detect visual effects of
- drugs, routine testing of NCEs prior to FIH is not recommended in the absence of effects in toxicology. However, the challenge for the future is to further characterise the relationship between drug effects on visual function in man and in animals to establish the predictive value of existing test systems and to develop novel assays.
- 8.10** Ototoxicity is a potentially important safety issue for drugs, but is hard to detect. Although some drugs are classically associated with ototoxicity, the extent of the issue is probably underestimated since the symptoms may appear gradually and are often indistinguishable from underlying disease processes. Any impact on hearing is temporary and reversible upon removal of the drug. Animal pharmacology models are available, but their utility in screening is yet to be determined as few mechanisms of ototoxicity have been determined and the forward prediction of the available tests is not established and can be insensitive.
- 8.11** Overall, the current model, where evidence of hearing impairment in toxicology studies or in clinical trials, is considered more appropriate. Nevertheless more attention should be paid to this under-reported safety concern. In toxicology and pharmacology laboratories, animal handlers and toxicologists should pay attention to behavioral changes in animals that could suggest an auditory dysfunction. Should these data, or emerging data in humans suggest further investigation of auditory function is warranted, BAER or histopathology of cochlea could be used for investigational studies in animals.

Chapter nine - *Immuno-safety pharmacology*

Current situation

9.1 The available methods to assess the interaction of drugs with the immune system do not meet all the needs for safety evaluation. Approximately 6% of drug withdrawals are ascribed to adverse immune responses, with a further 7% associated with bone marrow toxicity for which an immune response may be contributory. The difficulty with prediction of these responses is a consequence of their low clinical incidence, poorly understood mechanism of action, and the inability of non-clinical models to reliably identify hypersensitivity reactions in man.

9.2 Current methods for the non-clinical assessment of immunosuppression appear to identify the clinical risk adequately. For this purpose one or more of the following approaches are used: (a) examination of the histopathological appearance and/or weight of key lymphoid organs (thymus, spleen and lymph nodes), together with considerations of haematological parameters and of bone marrow cellularity, (b) tests designed to measure the functional integrity of the immune system in exposed animals. Of the methods available, tests for assessment of the integrity of antibody production (to either sheep red blood cells [SRBC] or keyhole limpet hemocyanin [KLH]), are commonly used, but other approaches (such as for instance immunophenotypic analyses) are also employed.

9.3 Methods, albeit poorly characterized, to identify host resistance are available and have been employed to assess defense against infectious micro-organisms or transplantable tumour cells, although the ability of these models to assess subtle effects is less confidently assured. The advantage of host resistance assays is that, in theory at least, they provide an holistic view of changes in susceptibility that may reflect compromised immune function. However, it must be appreciated that other forms of toxicity (such as, for instance, liver damage), and general ill health, may be reflected by changes in host resistance in the absence of a primary lesion in the immune system.

9.4 Functional assays; SRBC/KLH assays or immunophenotype analyses, particularly aimed at defining the quality of the immune response and the selectivity of changes in type 1/type 2 T lymphocytes, have been increasingly used to characterise adaptive immune function.

9.5 Methods for the identification and characterisation of chemical allergens have been developed and are widely used in safety assessments. Validated methods for the evaluation of contact (skin) sensitising potential are available. In addition, as yet unvalidated approaches have been developed for assessment of the potential of chemicals and proteins to cause allergic sensitisation of the respiratory tract and (in the case of proteins) gastrointestinal tract.

9.6 Approaches to identify compounds that induce autoimmunity, or to provoke idiosyncratic reactions are not available. These types of response will continue to be the most difficult to assess and predict. One general method that is available (in several guises) is the popliteal lymph node assay (PLNA). This has been, and remains still, the subject of investigations but has not yet been established as a reliable indicator of hazard. An understanding of genetic and environmental susceptibility factors will probably be the most fruitful area of research.

Challenges and opportunities

9.7 Although there are available methods that can be used for investigating the ability of xenobiotics to interact with and/or perturb the immune system, it is clear that they do not (at least as currently deployed) meet all needs of drug safety assessment. Thus, a common cause of attrition following launch of a new drug is the appearance of idiosyncratic drug reactions, often manifest at only low incidence, that are thought to have an immune/allergic pathogenesis. The ability of candidate drugs to provoke such reactions has proven extremely difficult to predict. There are a number of

factors that contribute to this difficulty, including: (a) the fact that such reactions normally occur only at low incidence, (b) there is frequently no attempt to define the mechanisms through which such reactions are provoked, and as a result there is uncertainty regarding the relevant immunobiological processes, and (c) it is likely that, as currently constituted, non-clinical toxicology and safety pharmacology studies are not appropriate for identification of chemicals that have the potential to elicit hypersensitivity reactions.

- 9.8** With respect to the last of these points, two separate but related potential deficiencies can be identified. The first of these is that appropriate assessments are not conducted. The second is that at least some of the assessments that are conducted currently may yield information of importance, but that the relevant questions are not addressed, nor the correct deductions made.

Proposals and recommendations

- 9.9** There is confidence that current non-clinical models are able to detect immunosuppression and impaired host resistance despite the validation of these methods being incomplete.
- 9.10** Methods to identify allergenicity have been widely used and for contact sensitization several approaches are validated. There remains a need to gain full acceptance of the methods available for detection of respiratory and gastrointestinal sensitizers. The local lymph node assay (LLNA) is a fully validated, OECD guideline method for the identification of chemicals that have the potential to cause skin sensitisation. The test is predicated on the fact that skin sensitising chemicals will provoke the stimulation of specific T lymphocyte responses in regional lymph nodes draining the site of exposure. This method therefore has the potential to identify chemicals that have an intrinsic potential to interact with the adaptive immune system, or which can be metabolically converted to such.
- 9.11** Other approaches developed initially for the assessment of industrial chemicals include methods designed to identify materials with the potential to cause allergic sensitisation of the respiratory tract: *the mouse IgE test* and *cytokine fingerprinting*. These tests are designed to define the quality of immune response that is provoked by exposure to a chemical allergen, and specifically whether selective type 1 or type 2 T lymphocyte responses are elicited. This approach allows determination of whether a chemical is likely to stimulate an IgE antibody response – the major effect molecule in many forms of allergic disease.
- 9.12** One potential innovation might be to consider the coordinated and structured application of the above methods, with or without incorporation of one or other version of the PLNA, to facilitate an holistic assessment of likely stimulation of an immune response. Used in concert these methods could provide: (a) an assessment of the inherent potential to provoke an immune response, and of the level of immunogenicity, and (b) an indication of the type (quality) of immune responses that will be elicited preferentially.
- 9.13** Approaches for the identification of autoimmune responses or idiosyncratic response have proven to be elusive and demand more intensive study and should include consideration of genetic susceptibility factors, polymorphisms and environmental factors (diet, overall health status, life style etc). Furthermore, it is pertinent to ensure that information relevant for a consideration of immune function from conventional toxicity studies, and an appreciation of cytokine expression patterns, are included in an overall assessment of impacts on immune status.

Chapter ten - Paediatric indications

Current situation

10.1 In most regions of the world, the majority of drugs used in children have never been tested in this target population. Moreover, they were approved only on the basis of data obtained in adults, either animals or man (although off-label use is high). Nevertheless, in both the United States and Europe, health authorities are encouraging paediatric studies by means of legislation such as the Best Pharmaceuticals for Children Act (2002) and the Pediatric Research Equity Act (2003) or the European draft legislation “Better Medicines for Children” (to be finalized, 2005). The aim of all of these considerations is to make valuable medicines available to children as soon as possible, addressing what is seen widely as an important medical need.

10.2 The need to extend the access of children into new therapies, and to develop paediatric formulations of existing drugs reinforces the urgency to implement strategies for early prediction of specific safety aspects in this population. In this context, the use of juvenile animals may be helpful and is usually considered on a case-by-case basis.

Challenges and opportunities

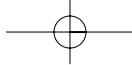
10.3 Traditionally, drug development in children has been performed once sufficient numbers of adults have been studied to define risks. Therefore, drug approval has not required the same level of evidence for paediatrics as it has for adults, given the approvals already secured on the basis of adult safety data. Evidence from paediatric development programmes recently, however, has suggested that children are dynamic and variable and reliance on adult data may underestimate the risks of the drug to children. Moreover, in the future, especially in the area of paediatric oncology, there will likely be an emphasis on drugs designed specifically

for children and adult human safety data will not be available as background to define risks.

10.4 Potential differences between adults and children include pharmacokinetic (e.g. clearance pathway) differences, and developmental differences in tissue structure and function, for example in vision and other senses, thyroid function, CNS plasticity and the haematopoietic system (with a spleen and thymus focus, rather than bone marrow in adults).

10.5 Clearly, non-clinical studies used to support an investigational new drug, or even a marketed drug for which paediatric use is desirable, may not directly address the concerns or the potential interactions of age, gender and the relative development of the various organ systems. Age- or gender-related kinetic and metabolic differences in response are not generally addressed. When performed, most studies in juvenile animals use neonatal rats, dogs or swine, assuming equivalent postnatal development to newborn infants.

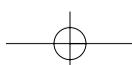
10.6 In Europe, a guidance document addressing the use of juvenile animals on safety assessment of paediatric drugs is being prepared by the Safety Working Party under request of the CHMP. A concept paper including the several items under discussion has been published in the EMEA website. FDA/CDER is in the process of developing Guidance to Industry on Non-clinical Safety Evaluation of Pediatric Drug Products. This document provides guidance on the role and timing of animal studies in the safety evaluation of therapeutics intended for paediatric use and when such studies might be needed. It is intended to serve as a general resource in testing and provide specific recommendations based on the available science and pragmatic considerations. There are no specific guidelines to address the use of juvenile animals for safety pharmacology studies of paediatric drugs, although there is a general statement in ICH S7A Safety Pharmacology guideline that consideration should be given to selection of relevant animal models, including age of animals.



Proposals and recommendations

10.7 Given the issues described above, the utility of safety assessment in juvenile animals is a subject that continues to be debated. Due to interspecies differences regarding the development of several organs and systems, the value of studies in juvenile animals to predict the drugs safety in children needs to be considered on a case-by-case basis, taking into consideration the information from studies in adult animals including reproductive toxicology studies.

10.8 This group proposes that the adequacy and appropriateness of all supporting non-clinical safety data (including safety pharmacology) should be considered as part of a paediatric investigation plan. Given the limited models available, the extent of non-clinical safety pharmacology testing to support paediatric clinical development will inevitably be decided on a case-by-case basis. Information from adult animal studies including reproductive toxicology should also be taken into account.



Chapter eleven - *Biotechnology compounds*

Current situation

- 11.1** Considerable experience in the development of biotechnology-derived compounds has been gained over the past 20 years. Clinical experience has revealed toxicity frequently not predicted from the initial non-clinical studies. The traditional safety pharmacology studies need to be used with care with biotechnology-derived compounds with the priority being assessment of functional indices of toxicity. The following areas should be considered:
- 11.2** *In vitro* testing for tissue cross reactivity and tissue distribution is essential in species selection and interpretation of pre clinical data.
- 11.3** Many humanized biotechnology derived molecules are immunogenic in animals. Animal species may produce neutralizing antibodies, animal anti-human response (characterized by increased clearance and for loss efficacy), toxic antibodies which may manifest responses disconnected from pharmacokinetics and anti-idiotypic antibodies that result in high unexplained exposure not correlated with pharmacodynamic/toxicodynamic actions.

Challenges and opportunities

- 11.4** Since prediction of immunogenicity of biotechnology-derived products in man is very poor, improvement of immunogenicity reduction strategies is necessary. Continuing progress in prediction, detection and prevention of harmful immune responses brings the promise of safer and more efficacious compounds in the future.
- 11.5** Considering that factors other than protein sequence are equally important for immunogenicity, and that immune responses are genetically determined and, therefore, highly individual, sequence analysis may not be sufficient to predict and avoid antibody formation although a number of companies

claim success in this area. Ex vivo T-cell activation assays and specialized animals models including genetically engineered mice and MHC defined primates are promising future directions.

- 11.6** Given the poor predictivity of the immune response the default has often been to use a primate model. It seems reasonable to challenge this premise and studies to establish its validity and explore whether non-primate species can be more widely used should be encouraged.

Proposals and recommendations

- 11.7** Increase human sequence content: Several approaches are pursued to minimize murine sequence content: chimeric antibodies comprising mouse variable regions and human constant regions; humanized antibodies in which murine CDRs are grafted onto a human framework and fully human antibodies produced by phage display or in transgenic animals. Nonetheless, antibody formation can occur with all these modifications. Abbot's Humira®, the first FDA-approved fully human antibody, elicits an immune response in 12 % of patients when administered without immunosuppression. Fully human proteins can be immunogenic, as immune tolerance can be broken under certain circumstances.
- 11.8** Improving solution properties: Protein aggregates are typically more immunogenic than dissolved proteins. Poor protein solubility can be overcome by optimizing expression, purification, formulation and solution conditions. An alternative method is to use rational solubility engineering.
- 11.9** Removing antibody epitopes: Antibody epitopes are often located at a small number of discrete sites on the surface of a protein. Modification of residues can reduce immunogenicity and binding of existing antibodies. Modified variants of Factor VIII and staphylokinase are examples.

11.10 PEGylation: The steric block of antibody binding by derivatizing the protein with polyethylene glycol (PEGylation) can decrease immunogenicity. PEGylation also increases solubility and often changes pharmacokinetic parameters which may permit less frequent dosing. PEGylation has been used successfully to minimize immunogenicity of enzymes. Protein design approaches may help to further optimize the balance between reduced immunogenicity, improved pharmacokinetics, and activity maintenance.

11.11 Identifying and removing class II MHC agretopes: The production of IgG antibodies responsible for clinically relevant immunogenicity can be minimized by identifying and removing class II MHC agretopes from the therapeutic molecule. The binding site of antigen-derived peptides at the class II MHC molecule and their binding specificities are well described. Following computational or experimental identification of MHC agretopes, a mutagenesis approach can be used to produce variant sequences that do not interact with MHC.

Chapter twelve - *Compounds selective for human targets*

Current situation

- 12.1** The development of sophisticated technologies to screen large numbers of chemicals has led to an increased use of human tissue derived targets. Consequently there is an increased likelihood that the drug candidates identified will be highly selective for human tissue/disease. While this may lead to more efficacious treatments it does give difficulty in species selection for non-clinical safety evaluation.
- 12.2** There is increasing evidence that around 50% of the toxicities seen with candidate drugs in development can be ascribed to the pharmacology (either primary or secondary) associated with the target receptor or protein. It therefore follows that an increasing number of projects will have compounds that do not cross react with the species commonly used for safety assessment. While studies in conventional species will provide information on intrinsic toxicity related to the chemistry of the compound a gap exists in understanding the evaluation of human selective or specific targets.
- 12.3** The issue of non-clinical safety evaluation of human specific compounds has been previously considered with biotechnologically derived pharmaceuticals. These have usually been large molecules that have been manufactured to replicate an endogenous human protein or are an antibody to a human specific target. Many of these biotechnology compounds were directed towards replacement therapy. The toxicity produced by these molecules has been mediated through the specific target receptors and selection of appropriate species has been imperative to understanding human hazard. Toxicity with the biotechnology compounds has so far been underpinned by an extensive literature supporting the understanding of the function of the specific target – examples being G-CSF, IL-1, IL-2, Erythropoietin and Insulin. For future targets that are exploited using small molecule chemistry it is likely that this prior knowledge will not be abundant. Furthermore the biotech

compound developed to date are frequently aimed at life threatening diseases whereas human specific or highly selective targets are now being identified for disease that are not life threatening.

- 12.4** In assessing the selectivity of a compound for a target two scenarios exist. The compound can have no cross reactivity with the animal target or cross reactivity can exist but the potency of the compound at the animal target is substantially less than for human. Pragmatically if the drop off is more than 20 fold it is unlikely that the dose levels can be achieved in an animal species that will enable sufficient assessment of pharmacologically associated toxicity.

Challenges and opportunities

- 12.5** Conventional evaluations will describe the safety pharmacology/toxicity associated with the chemistry of the molecule to be assessed but the following may be considered to assess pharmacologically related adverse effects:
- 12.6** Profiling in established laboratory species to confirm selectivity and selection of the most appropriate species.
- 12.7** Demonstrate that human specific molecules possess high enough potency for animal target.
- 12.8** Target distribution in normal and diseased tissue. Where targets are expressed in normal tissue it is worth considering its function if known to assess the potential deleterious effects of pharmacological modulation.
- 12.9** Sensitivity – in some instances the laboratory species may be less sensitive to compounds with high potency to human targets whilst having a similar physiological role. In such cases use of conventional high dose toxicology may permit establishment of satisfactory safety margins.
- 12.10** Transgenics – increasing access to transgenic models will in the future give many more

options to evaluate the human specific targets. Approaches that may have utility will include knock-out/knock in models where the endogenous animal gene is replaced by a cloned human gene. Information from straight knock out models may also be useful if the function of the gene in the animal model is assured to be similar in man. Where known, information from human populations with either malfunctioning or absent genes is particularly useful. An important caveat when considering knock out models is the potential to overstate the toxicity of inhibiting the target or vice a versa. In these circumstances it may be appropriate to consider use of a conditional knock out. It should also be established that the transgenic model functions in a similar way with the correct response elements operational when drawing conclusions on the utility of a transgenic model.

12.11 Homologous systems i.e. animal specific molecule to assess the pharmacology in a responsive animal species. Understanding the function of the target in both the animal species in comparison to man may also be important. With small molecules the option to use monoclonal antibodies for cell surface targets is also available and increasingly used. This approach should also consider the effect on regulation of the target. If the target is not a receptor approaches which target mRNA may yield useful information. Synthesis of an anti sense oligonucleotide may provide such tools but consideration of the chemistry associated with the oligonucleotide is also worthwhile.

12.12 Disease models – Toxicity and safety pharmacology are traditionally assessed in normal animals. It is conceivable that

understanding physiological response would be assisted by use of disease models. Experience with recombinant erythropoietin where hypertension was seen in patients and uremic rats despite no change observed in normal rats support this view. Furthermore, in situations where the target is expressed only in the disease situation (frequently seen with targets thought to be important in inflammation) the use of disease models may be helpful in establishing true therapeutic indices.

12.13 Statistically or mechanistically it may not be possible to reproduce all human idiosyncratic reactions in non-clinical studies. It is important to understand the strengths and limitations of such studies.

Proposals and recommendations

12.14 Evaluation of human specific targets can be expected to increase in complexity over the next 5 to 10 years. The understanding of similarities and differences between the human disease, animal model and responsiveness of the normal laboratory animal will increase substantially. It is therefore unwise to regulate this area until a much better understanding has been achieved. Precedence from the development of biotech large molecules should be used to guide each development programme individually with the selection of species and models justified rationally.

12.15 Where small molecules are directed towards human specific targets it will be necessary to fully evaluate the intrinsic toxicity associated with the chemistry and assessment of the pharmacologically related toxicity will need to be supplementary and rationally developed.

Chapter thirteen - *Education and training*

Current situation

13.1 Practitioners in the field of safety pharmacology derive from a variety of core disciplines. The majority are trained as pharmacologists, primarily non-clinical but some clinical whilst toxicologists, biochemists and biological scientists have all achieved success in the discipline. There is a strong integrative role of the safety pharmacologist in assessment of all the available safety data as well as their own specialty studies and also a need to relate to the expected clinical utility of molecules under investigation. It follows then that core skills need to be supplemented with continuing development and this, in the main, is provided in house by units providing safety pharmacology services.

Challenges and opportunities

13.2 The current trend to reduce practical training at undergraduate level is the initial fail point for science-oriented students who may wish to develop a career in pharmacology. Lack of opportunity to explore the range of science available in the field limits take up of further studies at Masters or Doctorate levels. Furthermore, facilities for Masters courses in this and cognate areas are minimal, largely driven by problems associated with animal experimentation.

13.3 Post graduate and postdoctoral training is also a problem, which various Professional Societies (notably the British Pharmacological and British Toxicological Societies) are trying to address in concert with industry. Whilst industry, notably the pharmaceutical industry, can and does maintain state of the art facilities and provides ongoing training for its staff, the academic base of the discipline is essential for both the wider good of the community but also to 'educate the educators' of the future.

13.4 Training has two elements: technical and interpretative and in the field of safety pharmacology lies across two domains. Data on

new medicines will be generated at the non-clinical level to permit the initial human dose. However, the clinical pharmacologist receiving the data must be able to relate to the science and the scientist to discuss the interpretative elements of the data set. Training, therefore, must be available to meet both types of need. The availability of such training is reducing in the UK and this was highlighted in the PICTIF report, in has been confirmed in various submissions from ABPI and Professional Societies to government and by the practitioners themselves.

Recommendations

13.5 The training provision for both non-clinical and clinical pharmacologists needs core support from government to help address the current limitations. Numbers of adequately trained clinical and non-clinical pharmacologists are falling which is a serious issue impacting on the performance of a major industry contributing to UK plc and to the underpinning academic base which supports it. New integrative Continuing Professional Development (CPD) courses are needed to use both non-clinical and clinical pharmacologists more effectively in the development of new medicines.

Value added and measures of success

13.6 Compound attrition related to adverse effects detectable in safety pharmacology studies will be reduced e.g. nausea and vomiting, CNS effects and latter stage attrition related to hepatotoxicity. In addition, increased tolerability may improve compliance increasing drug efficacy.

13.7 Although Phase I studies are very safe, in these study doses are escalated to identify the maximum tolerated dose. It is possible that in some circumstances tolerability issues prevent the achievement of exposures to predicted therapeutic levels that may be related to effects

detectable in safety pharmacology studies e.g. dizziness, nausea and vomiting etc. Therefore, improving safety pharmacology testing may reduce the Phase I attrition due to poor toleration in volunteers.

- 13.8** Investments in understanding further the relationship between prolongation of the QT interval and proarrhythmia may support the development of NCEs that prolong the QT interval that are not associated with causing TdP. This in turn will increase the 'chemical space' available for Discovery chemistry to exploit against novel targets thus negating the negative impact of hERG affinity. Furthermore, the incidence of late stage drug withdrawals related to arrhythmias will be reduced/eliminated.
- 13.9** Drugs selective for human targets are associated with specific challenges for the pharmaceutical industry. Improved testing strategies are vital in preventing the discontinuation of compounds and potentially loss of interest in targets based upon incorrect and misleading adverse safety pharmacology or toxicity derived from inappropriate models. The key value to gain from supporting

evaluation of human specific targets is an increased linkage between the target and the disease leading to more efficacious compounds in humans.

- 13.10** Further refinement of safety pharmacology studies (e.g. simultaneous telemetered recording of cardiovascular and pulmonary parameters and the application of PK/PD modeling) will reduce the numbers of animals used in non-clinical safety testing. This will contribute to the 3Rs.
- 13.11** Although compound attrition due to effects on the sensory organs appears to be low, improving non-clinical testing strategies may reduce further the incidence of adverse events related to the visual and hearing system. Furthermore such studies would raise confidence on the lack of drug effects on the sensory organs and any potential long-term consequences.
- 13.12** Through data sharing and increasing our knowledge of the concordance between non-clinical studies and human outcome would increase our confidence to make decisions on drug development.

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