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Meeting Report

Report on the International Workshop on the Use of Human *In Vitro* Liver Preparations to Study Drug Metabolism in Drug Development

held at Utrecht, The Netherlands, 6–8 September 1994

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The aim of this workshop was to bring together internationally recognized scientists engaged in the development and use of human tissue for drug metabolism studies so they might critically review the state of the art with regard to its applicability in drug development and safety assessment. The hope was to identify the principal limitations and problems involved in obtaining reliable and scientifically valid data, provide guidance for the proper use of human liver *in vitro* systems and interpretation of the results of such studies, and identify future research needed to overcome those limitations and problems.

No such workshop had been convened before although the use of human material for metabolism studies is increasing rapidly in some sectors and areas of the world. There is a desire in all sectors represented at the workshop—Europe, the U.S.A. and Japan—to apply these systems to drug metabolism studies to aid in drug development, clinical regimen design and safety assessment. There was, thus, a need to exchange scientific knowledge and information at the international level, a first step towards identifying and addressing the major issues for the use of such tissue, and this in order to begin to develop uniform guidelines for general acceptability of data and, hopefully, minimize the need for harmonization later at the international level. Such *in vitro* approaches also help to minimize the numbers of animals required in pharmacological and toxicological testing and, thus, also serve a useful ethical as well as economic purpose.

The following topics were discussed in detail: human tissue availability, preservation methods, quality assessment, methods of characterization, data variability, present applications, future developments, testing strategies and regulatory and ethical issues. As can be imagined, it was impossible to come to full agreement on all points. This report presents the principal observations made in analysing the current situation for each topic, followed by a series of recommendations as to how the utility of these systems might be achieved.

Human tissue availability

It was agreed that one of the major problems in advocating the widespread use of *in vitro* human testing is the relative lack of availability of suitable human liver material. The source of material is important—post-mortem sources are of little value, the material having to be as fresh as possible or suitably preserved. Sources of human material used in drug development are primarily "transplant reject" livers (i.e. livers that are unsuitable for transplant due to lack of a suitable recipient, injury to the tissues, poor anatomy, donor history or adverse pathology). Fatty liver can be used for the preparation of microsomes. Since fatty livers are often not transplanted, livers available for research are frequently fatty. Severely fatty livers are not suitable for studies with liver slices or isolation of hepatocytes. A correlation between fat content and tissue slice viability is needed. Cirrhotic liver is of little use for any preparation. Resected tissue is used when obtained at the onset of surgery as wedge biopsies and under conditions of minimal warm ischaemia. This tissue was considered by some participants to be of lower quality for metabolism studies.

In the U.S.A., national distribution networks such as the NDRI¶, the IIAM and the AHTU exist. These organizations are run on a non-profit basis and charge only an administration fee and expenses for supplying human material. The material is considered suitable as it is pre-perfused before dispatch and can be obtained in large quantities for processing and distribution. On the negative side, however, costs are high and there is little advance notice of tissue availability. IIAM in particular obtains "transplant reject" livers as described above. This material is delivered to IVT which processes and characterizes the material for P450 activities; subsequently, IIAM delivers slices, cells and subcellular fractions to the recipient's laboratory. At present, NDRI distributes tissue specimens directly to user laboratories, such as SRI International, which do their own tissue processing and characterization.

¶ Abbreviations: AHTU, Association of Human Tissue Users; COST, Co-operation in Science and Technology; ECOD, ethoxycoumarine *O*-deethylase; ECVAM, European Centre for the Validation of Alternative Methods; EGF, epidermal growth factor; FDA, Food and Drug Administration; HAB, Human and Animal Bridge Discussion Group; HPLC, high-performance liquid chromatography; IIAM, International Institute for Advancement of Medicine; ISSX, IVT, *In Vitro* Technologies Inc.; MRC, Medical Research Council; NDRI, National Disease Research Interchange; NIEHS, National Institute of Environmental Health Sciences; NTP, National Toxicology Program; TGFα, transforming growth factor α; UW, University of Wisconsin solution (or Belzer solution).

‖ UW and Euro-Collins solutions are the two solutions used for hypothermic preservation of organs before transplantation (for details see Olthoff KM *et al.* Comparison of UW solution and Euro-Collins solutions for cold preservation of human liver grafts. *Transplantation* **49:** 284–290, 1990).

AHTU also procures "transplant reject" livers, characterizes their P450 content and assesses viability in slice cultures, and distributes specimens directly to subscriber laboratories which are on standby for specimens when they become available. Surgical Research Institute of St. Louis University, which does not distribute tissue, obtains small amounts of biopsies from donor patients on a frequent basis and performs commercially sponsored research on site. The large procurement organizations (NDRI, IIAM, and AHTU) can receive approx. 100 livers/year. These are processed as above and each is accompanied by a complete medical history and is HIV- and hepatitis-tested. Delivery to the recipient is usually by cold storage in UW on ice. If subcellular fractions are to be shipped, they can be sent frozen in dry ice worldwide whereas cellular preparations require more careful handling. Intercontinental shipment is possible, however, if transport conditions are well controlled and do not exceed 24 hr.

In Europe, no such national network exists although a wide range of liver samples is available ranging from needle biopsies, which give only a small amount of material, to a few whole livers. Many local arrangements exist between research groups and hospitals for the supply of human material. The countries involved in the COST programme have proposed the formation of COSTNet—a network of European tissue banks. There is, however, a problem implicit in the potential use of such material for commercial purposes—there is no general agreement on the definition of "commercial use" within the EU and, thus, no agreement as to how the collected tissue can be used. The MRC in the U.K. has launched a pilot scheme to test the feasibility of maintaining a tissue bank and liver will be part of this study.

In Japan, human material for drug development and research is unavailable because of ethical concerns and legal restrictions. Some universities do have access to liver specimens for research purposes, but other Japanese groups must use frozen human material obtained from the U.S.A. or contract their studies abroad. Japanese scientists engaged in new drug development are hopeful that the ethical concerns can be resolved and the legal obstacles removed in the foreseeable future.

Tissue preservation

When human liver becomes available, there is often an excess of material which cannot conveniently be used all at once and in one place. The issue of how to preserve the material either before or after preparation becomes of vital importance. Owing to the scarcity of supply, it is essential that a great majority if not all available liver material be used and that this material be of serviceable quality. Two general methods of preservation were discussed: cold preservation (at 0–4°) and cryopreservation (at −70° or below). Preservation of the whole livers can only be achieved by cold preservation, preferably in UW solution, and up to 30 hr at 4°. As warm ischaemia for more than 10–20 min is detrimental to liver tissue, livers should be cooled as soon as possible after removal from the body. Wedge biopsies can also be stored at 4° in UW solution for up to 30 hr without significant loss of viability.

Several reports of successful cold or cryopreservation of liver slices and hepatocytes were given. Ordinarily, slices do not preserve for more than 2–3 days in cold storage and results can be variable. However, there is preliminary evidence for cold preservation of slices in a new, proprietary solution (AHTU) for up to 7 days without loss of metabolic function and that interlaboratory shipment of such tissue on ice is possible. Data supporting this claim have not yet been published. It was also stated that for shorter periods (a few hours) of cold preservation, the much cheaper Euro-Collins solution could be used.

Cryopreservation of human hepatocytes has been attempted by a number of groups using mixtures of penetrating and non-penetrating cryoprotectants with varying success. Human hepatocytes seem to be harder to cryopreserve and maintain viable than rat hepatocytes, and a number of groups experienced problems with attachment of hepatocytes and inducibility of drug metabolizing enzymes after cryopreservation. It was shown that when hepatocytes were isolated in good condition and with high viability, they attached better and were able to function better and for longer periods after cryopreservation if co-cultured with primitive biliary epithelial cells. Cryopreservation in alginate was suggested as a possible method for future evaluation.

Subcellular fractions have been cryopreserved without significant loss of metabolic activity for many years, usually in solutions containing 20% glycerol, and this was not considered a problem except that glycerol is known to inhibit cytochrome P4502E1. Owing to the lack of data and varying techniques and, in many cases, highly variable results with cold and cryopreservation of cellular material, however, it was agreed that further research was needed to assess: the length of time livers and cell preparations could be cold preserved; in what solution; and what was the best method of cryopreservation of whole cell preparations. This would best be achieved by a multi-centre evaluation of the published methods.

Quality assessment of preparations

Quality assessment was described as having two facets: (i) the quality of the liver tissue received at the laboratory, and (ii) the quality of the cell or slice preparation used in the experiments. There are no current methods for assessing whether or not significant deterioration in metabolic competence has occurred from the time of surgical removal of the liver to the time it is received in the laboratory. Some laboratories examine the tissue histologically to confirm the absence of disease or tissue deterioration but this is impractical as a quality control to determine whether or not to isolate cells or slices from that particular specimen. Training of hospital staff to cold-preserve the tissue quickly and store the tissue adequately is generally all that can be done at this time.

It was agreed that criteria for a successful preservation of liver material need to be established. It would be pointless to continue with a multi-centre evaluation as described above without a consensus on quality criteria. Viability and functional assays are required when whole cell preparations are used, whereas functional assays are sufficient with subcellular fractions. This was the subject of an intensive discussion at a workshop organized by the ECVAM: their findings have been issued as a report [1]. It was agreed that the criteria set out in the ECVAM report were worthy of consideration by this group. It was indicated that, in particular, the viability criteria recommended in the ECVAM report were acceptable.

After cells or slices have been prepared, all laboratories conduct one or more assays to assess quality—either viability or functional assessment or combinations of both. There was no consensus on the best assays to use and this needs to be resolved before any multi-centre evaluation as described above can be initiated. Some critical observations were made, however. Freshly isolated cell preparations exhibiting greater than 75–80% viability (assessed by trypan blue exclusion) are acceptable for experimentation. Preparations with lower viability are salvaged by Percoll gradient centrifugation in some laboratories but this may lead to selection of particular cell populations. While initial trypan blue exclusion is a useful, convenient method, it was generally agreed that it does not offer a reliable indication of human hepatocyte survivability in suspension cultures and that viability at the end of the culture period should also be assessed. It was also generally accepted that cell attachment was a much more reliable indicator of cell quality. If hepatocytes attached to culture substrata, one

could proceed with a metabolism study with confidence that the cells would retain functional competence during the short incubation period. This can be important in determining whether to proceed with large-scale studies at the risk of wasting expensive test materials in an experiment that would need to be repeated.

It was also agreed that at least two different kinds of measurements of cell quality should be performed: a viability indicator which is quick and easy to perform (such as trypan blue exclusion, lactate dehydrogenase leakage, maintenance of K^+ gradient, or the MTT assay) and a cytochrome P450 enzyme indicator, which can be more time-consuming. ECOD (a fluorescence assay) is often used but a specific measure of cytochrome P450 3A4 was thought important as this is a dominant cytochrome P450 form in human liver. Data presented on ECOD measurements in different laboratories have shown wide variations and relative inconsistency in activity of cold-preserved slices over 24–48 hr, raising concern as to whether the cold preservation methods used still had shortcomings.

It was agreed that a small working party should be set up to define the quality assessment criteria and preservation criteria in more depth and propose appropriate guidelines for their use.

Characterization methods

Once the liver preparation has been obtained, it needs to be characterized. It was agreed that the preparations need to be phenotyped for cytochrome P450 profile using selective (or, preferably, specific) probe substrates and specific antibodies (such as anti-peptide antibodies to the open loops at the C-terminus of the protein). Some "specific" antibodies may not be, for example, antibody against CYP1A2 may also detect CYP1A1 although separation of human CYP1A1 and 1A2 by Western blotting was shown. Specific chemical inhibitors may also be useful. It would seem to be important to choose the concentration of substrate and/or inhibitor carefully to avoid non-specific effects and pick up only the intended isoenzyme form. Functional criteria, such as carbohydrate, protein, urea and lipid metabolism, can also be assessed reasonably rapidly and are occasionally used to characterize hepatocyte preparations but these may require extra time and not be needed for drug metabolism studies.

Some of the assays for cytochrome P450 such as testosterone hydroxylation (an HPLC assay) are relatively time-consuming. Others, like ECOD, can be performed more quickly; ECOD may also be useful as a characterization marker as it has proved to be for viability although it is not as rapid as the other viability markers (e.g. trypan blue exclusion, MTT assay). Phase 2 markers are more difficult but 4-methylumbelliferone glucuronidation may be a possibility. Responsiveness to hormones (e.g. glucagon-induced changes in glycogen content) may be important if preparations are to be used for this purpose. It was pointed out that few, if any, markers of cytosolic enzyme activity are included. This may be a problem as leakiness of the cells following preservation may lead to selective loss of these enzymes or soluble cofactors. From the industrial perspective many of these tests are too cumbersome and there may be different objectives for assays using subcellular fractions and whole cell preparations. Subcellular fractions can be stored for an extended period of time and characterization tests can thus be performed at leisure. For whole cell preparations, the tests must give an answer quickly so that a decision can be made as to whether the preparation can be taken further, and used. Industrial laboratories need rapid, reproducible and robust assays.

Since a working party set up by ECVAM to recommend a series of probes for use in characterizing liver preparations will report in the near future, it was recommended that no working party be set up at this point.

Data variability

Data variability in drug metabolism within the human population is well recognized and can be a result of environmental differences as well as genetic polymorphism or other intrinsic factors. Data variability is, thus, the second most important problem in extracting useful information from human *in vitro* liver preparations. A number of participants presented illustrative examples of variation in data obtained using human liver preparations. Kinetic parameters measured in specimens can range from 3- to 30-fold, depending on the cytochrome P450 isoenzyme involved. A correlation of metabolic data *in vivo* with the amount of CYP2C9 accounted for a 10-fold difference in metabolism among samples. The inducibility of CYP3A4 by FK-506 was also shown to be qualitatively variable as was the responsiveness of cell samples to EGF and TGFα. The media used for culturing hepatocytes/slices and performing the assays was also identified as a possible factor in variability of data.

It was agreed that variability due to the intrinsic nature of the sample must be considered acceptable as long as it is characterized (as is variability in human subjects used for testing). This, indeed, may be a useful tool but any extrinsic variability due to technical factors must be eradicated. It was pointed out that inter-laboratory variability could be a problem, with even the assay of protein content showing a 200% variation. This may be due to the use of non-standardized assays. It was agreed that validated, standard protocols need to be published—this could now be done for assays involving subcellular fractions and, perhaps, liver slices, but not yet for isolated cells because, although there exist published methods, they are more varied. It was further agreed that a working group could be set up to begin putting together standard protocols although it was recognized that this was a huge task and would best be performed in collaboration with bodies already beginning to look at standardization (e.g. ECVAM and the Committee on Regulatory Affairs of ISSX).

Present applications

This section of the workshop examined what is currently being done in the field of drug metabolism for drug development. The following kinds of studies were being conducted with human liver *in vitro* preparations at the time of the workshop:

The screening of analogues for metabolism (M,H,S)
Metabolic profiling (H,S)
Rates of metabolism (M,H,S)
UDS assay (H,S)
Mechanistic studies (H,S)
Co-culture studies (H,S)
Drug–drug interactions (M,S)
In vitro/in vivo pharmacokinetic studies (M,H,S)
Physiological studies—effects of hormones, growth factors and cytokines (H)
Formation of toxic metabolites (M,H,S)
Comparative metabolism (M,H,S)

M, microsomes and other subcellular fractions; H, hepatocytes; S, slices.

It was agreed that the choice of the preparation to be used in metabolism studies was determined to a large extent by the study's objectives. Hepatocytes and slices are most generally useful and valid models and superior to subcellular fractions, for generating metabolic profiles. Microsomes, S9 and cytosolic preparations are useful for screening for primary metabolites and are more economical where many incubation parameters (e.g. kinetics) or large numbers of specimens are to be studied. Techniques for microsomal studies are well established and are more readily subject to standardization and acceptance of kinetic data, although many kinetic studies with hepatocytes and

slices have been published. With regard to the use of slices, the issue was raised that metabolism seems to be a function of slice thickness. Data was presented showing that monolayer cultures of human hepatocyte gave metabolic rates greater than those found in slices. Explanations for discrepancies between slices and hepatocytes may be due to: inappropriate slice culture leading to less than optimal O_2-penetration to internal cell layers; and/or more active subpopulations of hepatocytes attaching to the culture dish; and the more heterogenous population of cells in slices compared to hepatocyte cultures. These discrepancies must be resolved before either human *in vitro* liver system can be used successfully in human physiologically-based pharmacokinetic models.

Development work

This section covered the most important new uses of human liver material in the foreseeable future, assuming that availability problems can be overcome. Three areas were highlighted: drug–drug interactions, cytochrome P450 induction and pharmacokinetics/toxicokinetics.

Drug–drug interactions. Drug–drug interactions in humans that result in impaired effectiveness or produce adverse effects are one of the most important concerns in drug development and need serious investigation before a drug can be released for use. The use of human microsomes can show some inhibitory interactions but this depends on the kinetics of drug–enzyme interactions. An *in vitro* whole cell system would be better than subcellular preparations at predicting inhibitory drug–drug interactions such as mutual inhibition by two substrates for the same enzyme, although there are some instances where the two systems give comparable results (e.g. taxol). In this case, the K_m for the metabolism should equal the K_i for inhibition and, thus, the respective possible concentrations of the drugs *in vivo* being known, the interaction can be predicted. This was illustrated using the interaction of isoniazid and phenytoin, where metabolism saturation was predicted as was the large increase in phenytoin plasma concentrations in the presence of isoniazid as well as subsequent toxicity. Mechanism-based inhibition (suicide substrates or metabolite inhibition) can also be studied using human liver preparations. It was suggested from an industrial standpoint that these studies, which require considerable effort, should only be performed once the decision has been taken to put the drug into man. Microsomal data using probe substrates and other common drugs with known metabolism were considered adequate to get an IC_{50} value for many interactions but there were instances when microsomes did not give the correct answer. Cell lines transfected with specific cytochrome P450 genes can be used for reaction phenotyping and thus give confirmation of drug–drug interactions. These kinds of studies may be predictive but caution should be used in assessing the contribution of a particular enzyme to total metabolism.

From a regulatory point of view, the importance of drug–drug interactions and the precise moment this information would be useful were discussed. In some cases microsomal data are sufficient and the advantage of a whole cell preparation may lie in its being a first exploratory experiment in a human system.

It was concluded that microsomes are useful for showing drug–drug interactions and that hepatocytes and slices can also be used and, indeed, show some drug–drug interactions which are not uncovered by microsomal experiments.

Cytochrome P450 induction. The induction of cytochrome P450 can only be examined in whole cell preparations and can be considered a special form of drug–drug interaction. Hepatocyte cultures appear most suitable as potential models at this time although slices have been used. Human preparations are reportedly more phenotypically stable in culture than those from rat. It has proved difficult to show induction of cytochrome P450 in cultured hepatocytes although many modifications of culture conditions have been tried (such as the use of extracellular matrices, DMSO and other media additions). Some data with whole cell preparations on the effects of rifampicin and omeprazole (good inducers in human but not rat hepatocytes) and the effects of methylclofenapate on lauric acid hydroxylase induction indicated progress. In some cases a simple culture system could show induction but more often a more sophisticated system (e.g. co-culture with primitive biliary epithelial cells) was needed. It has been demonstrated, at least with families 1A and 3A, that CYP enzyme induction in primary human hepatocyte cultures was related to an increased transcriptional activity of the corresponding *CYP* gene. This dispels the notion that the enzyme activity was simply preserved and not induced. Although routine use of hepatocyte cultures to assess induction of cytochrome P450 has not been fully demonstrated yet, progress has obviously been made, and it was recommended that further work was needed to establish the model for this purpose.

Toxicokinetics/pharmacokinetics. In this section the question of whether *in vitro* kinetic data from human liver preparations can be used to predict *in vivo* pharmacokinetics or toxicokinetics was addressed. Data showing that various models of interaction of the drug with the liver cell could be used to predict pharmacokinetics were presented, but the differences between the models were often small and all predictions fitted in well with *in vivo* data. There were examples, however, of a 10-fold deviation from predicted pharmacokinetic constants following use of the calculated scaling factors. A number of possible reasons for these deviations were discussed: differences in membrane permeability of the substrate and/or metabolites which led to good correlation at low permeability but poor correlation at high permeability. These factors may be related to the unstirred water layer around cells *in vivo*, differences in blood/tissue distribution *in vivo* leading to microsomes giving a good correlation with low clearance drugs and cells with high clearance drugs, binding of drugs to plasma proteins and active uptake of drugs.

Data were presented showing that the pharmacokinetics of furan and several other compounds could be accurately predicted from kinetic studies with isolated rat hepatocytes, suggesting that isolated human hepatocytes could be used to predict pharmacokinetics in people for drugs where the liver was the major metabolizing organ. Kinetic studies with isolated hepatocytes can theoretically be used to predict when hepatic clearance will be flow-limited and when first-pass metabolism will be important for new drug candidates.

The importance of cell culture medium composition in maintaining the biochemical homeostasis of isolated hepatocytes was discussed. Nutritive media containing essential amino acids and cofactors rather than simple buffers or balanced salt solutions should be used. Isolated hepatocyte systems can also be used to investigate drug toxicity under physiologically appropriate experimental conditions. The importance of integrating pharmacokinctics into the design of *in vitro* toxicity studies was discussed. Evaluating drug toxicity *in vitro* using concentrations and exposure times that reflect exposure of the liver *in vivo* is more likely to lead to accurate predictions of drug hepatotoxicity *in vivo*.

There are, thus, many problems still to be sorted out with regard to the use of *in vitro* human liver preparations for pharmacokinetic/toxicokinetic studies, although it was agreed that this is a very important area of study which needs to be explored in detail.

Testing strategies

The main focus of this workshop was on the potential use of *in vitro* human liver preparations for drug metabolism studies in drug development and, as such, the industrial perspective on how these methods are, and can be, used

is of paramount importance. Different companies are pursuing different testing strategies in terms of the importance and timing of human liver tissue preparation studies in the drug development process. There appears to be fairly widespread use of *in vitro* systems, mostly at the subcellular level, and to a lesser extent, of hepatocytes and slices (depending on the availability), in exploratory and later developmental stages (after lead compound identification) depending on the company. These methods were said by industrial participants to provide information and guidance for clinical studies, reduce drug development time and cost as well as the number of animals used in drug development. The most important objectives cited for these studies were: (1) to identify the enzyme(s) involved in metabolism of the drug and, thus, potential interactions with other drugs and with endogenous metabolism in addition to the effects of genetic polymorphisms in the human population and possible differences in drug clearance; (2) to identify any induction of drug metabolizing enzymes; and (3) to identify any drug metabolites that might be found in man so that potential toxic metabolites could be singled out and a suitable species for toxicological evaluation chosen. In effect the species chosen for toxicology is limited to rodent and dog (though other species are sometimes used). If no suitable toxicological species can be found, the identified potential toxic metabolite in man (seen in incubations with human liver material) can then be administered to the species chosen.

Companies would like to have access to a constant source of tissue from a well-characterized liver bank that includes hepatocytes and/or liver slices (when methods are validated and this becomes possible). Currently, existing banks are limited to microsomes and subcellular preparations and to liver pieces from which those preparations can be made after thawing. The characterization of these tissues would be done using probe substrates, inhibitors and antibodies as described above (although it was noted that there are probably more and better probes available in industry than those being currently discussed and that these need to be shared with the scientific community at large). It was noted that a number of companies were on the point of offering drug metabolism assay kits on a commercial basis. Cells transfected with single enzymes are also used for specific purposes and the use of immortalized hepatocytes will be started as they become available. This may circumvent the problem of availability of human material if such immortalization proves successful. What the pharmaceutical industry needs most is fully validated methods in terms of predictability, reliability, reproducibility and cost effectiveness. There is also a need for timely and adequate supplies of human liver material to be used during the research and development phases of a new drug.

Regulatory and ethical issues

It is obvious but worth stating that the value of metabolism data from human tissue *in vitro* studies will be enhanced considerably for clinical applications as well as for drug development if the bodies regulating drug acceptance in Europe, the U.S.A. and Japan are willing to accept data from such studies. The position of the FDA was stated to be that *in vitro* human liver studies give useful information which is more meaningful than animal data in a cost-effective and rapid manner. There was increasing interest and encouragement in submitting such data when available and it was noted that other regulatory agencies besides the FDA have exhibited interest in receiving such data. There is, however, only a limited data set from which to show *in vivo/in vitro* correlations. The limitation of extrapolation and interpretation is governed by the limits of the available knowledge on the subject. There are no set requirements for human *in vitro* data. For this type of data to gain greater acceptance, more data on *in vivo/in vitro* correlations will need to be presented and the relevance and reliability of such data will have to be established.

The NTP of the NIEHS in the U.S.A. was one of the foremost groups in providing funding for research on redevelopment and evaluation of human liver *in vitro* systems for comparative metabolism studies with an aim to improve human risk assessments of environmental chemicals. More recently they have been given the task of developing assays aimed at the reduction of animal use in research (based on the NIH Revitalization Act, 1993). Human liver slice studies are one of the areas of research where exposure to mainly environmental chemicals are being examined. Such studies, unlike studies on potential drugs, cannot be performed on human volunteers. The long-term goal is to accumulate permitting the extrapolation of animal and *in vitro* human data to human risk assessment, to develop and validate assays and protocols to reduce animal use in safety testing and to establish criteria for validation and regulatory acceptance of alternative testing. Current research uses liver slice cultures, human cell assay systems and transgenic cell lines and publication of results is encouraged.

The regulatory authorities, at least in the U.S.A., are thus inclined towards using *in vitro* human liver preparations in drug development and would welcome its introduction on a broader basis but first require data confirming the relevance of the assays.

From an ethical point of view, however, some serious problems exist, particularly in Europe and Japan. In Europe, the lack of consistency in regulations governing the use of human material between individual countries is a severe drawback—a framework exists for the free movement of transplant material in the EU but this is not the case for research material. Indeed, it is likely there would be a public outcry in some EU countries (e.g. the U.K.) if rules governing transplantation of human material were extended to cover use of such material for research or testing purposes. The idea of extending legislation designed to regulate the use of human material for transplantation to its use for research and testing was not, however, rejected by the participants but would need to be presented clearly to the European public and debated openly on an ethical, economic and scientific plane. It was suggested that the following proposal could be put forward: any material unsuitable or not needed for transplant could be used for research and testing under a licensing scheme similar to that used to regulate animal experimentation and suitable collection, storage and distribution networks could be set up. A group within the Commission of the European Union is currently examining the problem of medical ethics including the use of human material and it was agreed to await the outcome of this discussion before moving on the ideas expressed above.

In Japan, the situation is, if anything, more difficult, given the legal and religious opposition to the use of human material in scientific research and testing. The HAB (a group primarily comprising members from academia and industry) has been formed to facilitate initial discussions of eventual international guidelines for the use of human material in *in vitro* metabolism studies (amongst other things). The group needs to be acutely aware of public opinion and must move forward slowly and on the basis of consensus. HAB aims to discuss the use of human material with the Ministry of Health and Welfare, establish a non-profit making organization for the collection, storage and distribution of human material (as in the U.S.A.) and arrange for the licensed use of human material. It is hoped that the HAB Foundation will be founded in 1995–1996 to set up a tissue bank, a databank and a research facility.

The ethical issues are, thus, formidable and in some sectors dictate regulatory involvement. They need careful,

skillful handling. A public debate needs to be initiated to gauge the state of public opinion on these matters.

Conclusions

The use of human liver as material for drug metabolism studies is an optional choice as an experimental approach to providing data to facilitate and improve new drug evaluation and development. Many pharmaceutical companies are presently applying available methods and that number is increasing. However, there are recognized and serious problems with this approach. The major problems are:

(1) Tissue availability (particularly in Europe and Japan).
(2) Difficulty in storing the material obtained so as to make the most efficient use of resources.
(3) The lack of uniformity and validation of methods used.
(4) Data variability.
(5) Quality assessment criteria to ensure data acceptability.
(6) Ethical considerations associated with the use of human material.

The singularly relevant nature of human tissue for providing information on many aspects of drug metabolism in humans (such as drug–drug interactions, enzyme isoform identification for estimating interindividual variability, enzyme induction and prediction of *in vivo* pharmacokinetic/toxicokinetic parameters in advance of clinical testing) are very strong reasons for attempting to solve these problems. To this end a series of recommendations was compiled to move the accepted use of human material for drug metabolism studies forward.

Recommendations

As agreed the recommendations fall into three categories: those areas on which agreement was reached; those areas on which further work is necessary; and those that require further debate and discussion.

Areas agreed upon

(1) Warm ischaemia should be avoided at all cost when harvesting liver material (<20 min) but cold ischaemia is acceptable in UW solution for up to 30 hr (or for shorter periods in Euro-Collins solution).
(2) Human liver *in vitro* methods are used at present to examine routes of metabolism and drug–drug interactions. Continued validation of these methods for *in vivo* extrapolation and human hepatocytes for induction of drug metabolizing enzymes is useful.
(3) The criteria for viability recommended by ECVAM are applicable and may be employed. The ECVAM recommendations on functionality testing should be reviewed for their applicability to human liver preparations.

Areas for further work

(4) Further research is required in the areas of preservation of human liver material (e.g. how long can preservation be extended? What are the optimum conditions for cryopreservation of human liver material?).

Areas for further discussion and debate

(5) A working party should be set up to devise an agreed scheme for quality assessment criteria and a set of protocols for quality assessment.
(6) A second working party should be set up to devise an agreed scheme for preservation criteria for the various types of preparations. These working parties could be made up of participants from this workshop together with invited experts.

Representation should be inter-regional

(7) Working groups should be set up to write agreed, validated standard protocols for assays to be used in drug metabolism studies. These groups should work together with groups from other organizations (e.g. ISSX) towards a common goal. The preparation of protocols for subcellular fractions could be undertaken first as this is most advanced.
(8) An open, public debate should be initiated on the issue of the use of human material for drug development and testing and the possibility of legislation to facilitate this use.

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