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Features and Prospects of the MEIC Cytotoxicity Evaluation Project

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Summary

The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) is an international programme organised by the Scandinavian Society for cell Toxicology. The program started 1989 and will end in 1994. As an update of the original, detailed 1989 description of MEIC, this paper presents the actual aims, main assumptions, design and evaluation methods of the study. The present status of the testing activities and the prelinimary evaluation performed are accounted for. Finally, the prospects of MEIC are discussed. The study may contribute to a future reduction of animals in toxicity testing. Results of the study could also lead to a more rationale science of toxicology, based on the reactions of cells and cellular receptors, rather than on the reactions of animals.

Introduction

In the last two decades, a new methodology has appeared in toxicology, i.e. *in vitro* toxicology. It consists of methods to study toxic action of chemicals to human targets in test tubes, rather than in animals. The targets may be cultivated cells, isolated receptors, cell organelles, bodily macromolecules or extracellular transmitters or hormones. Toxicokinetic *in vitro* studies as well as QSAR-analysis based on test tube data may also be included.

In vitro methodology has been successfully applied in two areas of toxicology, i.e. mechanistic studies of toxicity and biotransformation as well as in testing of substances for mutagenicity and carcinogenicity (1). The use of *in vitro* methods in a third field of toxicology is developing since ten years, i.e. the use of in vitro tests for general toxicity, such as acute systemic toxicity, target organ toxicity, local irritancy, and teratogenicity. Recently, a wide array of *in vitro* assays of various types of general toxicity has been developed. These have not yet won general acceptance, however. There are several reasons for the lack of general acceptance of *in vitro* tests for testing unknown substances on general toxicity (1). One reason is that these tests usually lack a mechanistic basis, i.e. cells and other test tube targets are used analogous to laboratory test animals. Another reason is the complexity of general toxicity, being a gross effect of chemical action to different targets combined with many toxicokinetic factors. A third reason is the difficulties involved in the necessary validation of such tests, that is the process to establish the relevance as well as the technical reliability of a test (2, 3). Indeed, it is almost a "mission impossible" for one laboratory to raise funds and energy sufficient for a confirming validation of one method (3).

To promote the development and, especially, the practical use of *in vitro* methods as primary tests of quantitative, general toxicity of various types, several organisations have recently starged multicenter validation programs (2, 3). There are mainly two types of multicenter validation programs. The first type is a reliability-focused study, by which one or a few in vitro tests are used in several laboratories to test the same reference chemicals to establish variability of the methods over time and between laboratories (3). Relevance of methods may also be evaluated by comparisons between *in vitro* and *in vivo* toxicity. The second type is a relevance-

focused study, which primarily evaluates relevance by tests of the same chemicals in many in vitro systems, to sort out the best test or combination of tests by in vitro and in vivo comparisons (3). The most relevant methods may then be evaluated for reliability. Well designed ongoing and future relevance-focused multicenter validation studies have a real chance to introduce *in vitro* assays in general toxicity testing, provided that the tests themselves are good enough or can be made good enough. Such studies are economical and feasible, since an almost unlimited number of methods can be evaluated parallelly against a single database of in vivo toxicity(3).

FRAME (British Fund for Replacement of Animals in Medical Experimentation) started relevance-focused multicenter validation already in 1982, and was later followed by other organisations (U.S. Soap and Detergent Association, U.S. Cosmetics, Fragrances and Toiletries Association, French Ministry of Research) as referenced in previous papers (1–3). This paper will present an updated description of the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC), a five-year, relevance focused international multicenter validation programme, started in 1989 by the Scandinavian Society for Cell Toxicology (SSCT).

A brief outline of MEIC was described already in 1987–88 (4,5). The original programme was described in detail 1989 (1). However, MEIC was planned as a flexible project. Thus, the original program (1) has been modified and also enlarged with time. Also some preliminary results have emerged. As an update, the present article will supplement the original description (1). Thus details in the original paper will only be summarized, while the emphasis is put on new developments.

Basic outline

A list of 50 nonselected chemicals was presented in 1989 (1). All interested international laboratories wre invited to test these chemicals in their own *in vitro* assays of various types of general toxicity and toxicokinetics. Laboratories must start with chemical No. one, and then proceed with tests according to the order of the dhemicals in the list. Results should be submitted successively to the MEIC committee, which then evaluates the relevance of single and combined results for various types of human toxicity (acute and chronic systemic toxicity, target organ toxicity, local irritancy to the skin, eye, etc.). Evaluation is made step-wise, so that different segments of the list (no. 1–10, 11–30 and 31–50) are evaluated successively, at a pace determined by submission of results for the different segments by a majority of laboratories. Evaluation is made quantitatively, with use of human reference data. In vitro toxicity data are combined with toxicokinetic data to model human toxicity. Animal data (LD50, Draize tests, etc.) for the chemicals are compared with the human reference data, also. In that way a baseline of animal test prediction of human toxicity is created, which can be used to judge the effectiveness of *in vitro* tests.

The program will be finalized in 1984. The primary aim is to be able to sort out the best combinations of in vitro tests predicting acute toxicity, chronic toxicity, skin irritancy, etc. Before such batteries are recommended for practical use, tests included in the batteries will be evaluated for their technical reliability, as a last phase of the programme (if the reliability was not known before).

Assumptions and hypotheses

The first assumption underlying MEIC is that various types of human toxicity indeed might be modelled by *in vitro* toxicity data, in most cases combined with *in vivo* or *in vitro* toxicokinetic data. A modelling in three steps has been described (1). 1. *In vitro* tests of a chemical on cell lines, organ-specific cells, and various extracellular receptors, resulting in a series of inhibitory concentrations for important human bodily targets. 2. Knowledge of, or *in vitro* tests for, toxicokinetics, resulting in a series of actual tissue concentrations in

different compartments/organs of the body of a chemical at known exposure. Also knowledge of the relative vital importance of the different tissues. 3. The final step would be to insert the first set of data into the step-two toxicokinetic model, to predict a first-hand toxicity, quantitatively and qualitatively.

The second assumption involved in MEIC is the basal cytotoxicity concept (1), which claims that general toxicity of chemicals most often is interference with basal functions shared by all human cells (6). Other types of toxic mechanisms (organspecific cellular toxicity and extracellular, organisational toxicity) would, according to this concept, be relatively rare effects in man of non-selected chemicals. Basal cytotoxicity might be tested on economical and animal-free cell lines, preferrably of human origin. If cells are cultured under physiological conditions the basal cytotoxicity tests would, furthermore, not lead to any false positive results, since man is sure to succumb if basal cytotoxic concentrations is reached in any vital organ/tissue.

If this basal cytotoxicity hypothesis were true, future batteries of *in vitro* tests for general cytotoxicity would be simplified, i.e. a large number of organ-specific systems would not be necessary to cover the majority of target organ effects. These would, instead, be predicted by knowledge of basal cytotoxicity and distributional factors (1, 6).

A third assumption has evolved during the progress of MEIC, and has only been discussed before in a validation article (3). Probably, a future *in vitro* modelling of human toxicity will be more economical than the conventional animal testing, because a relatively low number of different *in vitro* tests may be combined in various ways to predict the different types of toxicity. By the choice of in vitro tests of minimal complexity to model human toxicity, information on new types of toxicity will require less testing each time (another in vitro test is just combined with old tests-like hirigana characters), while the unique features of the animal experiments necessiate a new complete test for each "new" type of toxicity (like kanji characters). To promote rational, future in vitro testing, MEIC avoids sorting out specific tests for each purpose. This would unnecessarily impose the unique animal test features on new tests. Instead, *in vitro* results will be evaluated as components of models of the various types of general toxicity. Hence, MEIC tries to select a minimal number of tests needed to predict most toxicities.

An example may illustrate the assumption. Let us say that 5 factors may roughly predict acute lethal dosage for most substances: a, acute basal cytotoxicity; b, absorption in the intestine; c, distribution in the body, measured *in vitro* as permeation of cell barriers and absorption into cells, lipids, etc.; d, permeation of the blood-brain barrier; e, biotransformation in the liver as measured in co-cultures of liver and target cells. Possibly, acute eye irritancy may then be a function of a, b and c, plus more factors (such as pertubation of corneal proteins). Acute skin irritancy would likewise be a function of a and b, plus permeation of epidermal skin. All types of chronic toxicity (systemic and local) could well be a function of the above-mentioned factors, plus new specific data (chronic basal cytotoxicity, excretin from the body, etc.). To get information about new types of toxicity, one would thus just add a few data to the knowledge already aquired, while new animal tests cost as much, or moer, than foregoing tests.

Aims of MEIC

ther project has several goals (1). One is to chart the areas of possible application of *in vitro* tests of general toxicity. This includes a comparison of the effectiveness of animal tests and *in vitro* models, for the same set of chemicals. Another aim is to propose suitable batteries of in vitro tests, in such cases when these are better than animal tests. Note, that also a negative outcome of the evaluation is valuable-if the study can not demonstrate the effectiveness of *in vitro* tests for a purpose, the

continued use of traditional animal tests has been better funded from an ethical standpoint. A third aim is specifically to prove or conterprove the assumptions made. A fourth aim is to increase knowledge of cellular and toxicological processes by the unique comparison for the same set of chemicals of various *in vitro* and *in vivo* toxicity data-this is basic toxicological research beyond the primary pragmatic aim to sort out good in vitro tests. With time, the larger part of this research will probably be performed independently by participants, rather than by the committee.

Design of the project

The programme is organized by the Scandinavian Society for Cell Toxicology. A committee elected by the Society for this purpose include five experienced cell toxicologists, plus one expert on computerized statistical modelling and one expert on pharmacy. To communicate with participants a MEIC Newsletter has been issued four times per year since the start 1989. Also, yearly meetings for MEIC participants are arranged in Europe, the U.S.A. and Japan, respectively, always in connection with large toxicology or *in vitro* toxicology meetings. Compared with other validation programmes (2, 3) MEIC is ceed around the needs and judgements by participants-participants themselves determine which methods are to be evaluated and also set the pace of the testing. Therefore good communication is essential. During the three years since the start of the programme 80 laboratories from all around the world have joined the study. New participants are still welcome at the time of writing this, but with time new participants will have difficulties in submitting blindfolded *in vitro* results, for some types of toxicity (acute systemic toxicity and skin irritancy). Since most participants usually contribute with more than one assay, over 200 test systems are now used in the study. Most of these systems are cytotoxicity assays (animal cell lines, animal organspecific cells, human cell lines, human organospecific cells, such as liver cells, keratinocytes, lumphocytes, etc.). Many systemsare toxicity tests to yeast, plant cells, marine invertebrates, etc. Some laboratories participate with organ cultures, such as artificial skin. Other laboratories contribute with mechanistic studies, toxicokinetic systems, or physico-chemical data. Any test is welcome to the study, if it is considered by the participant to have any relevance to general toxicity in man. Also contributions of in vivo data for the substances are welcome.

The selection of the 50 chemcals was made by the Poison Information Centre in Stockholm, Sweden. The criteria for selection were 1. known data on human acute lethal blood concentrations and dosage, and 2. known mouse and rat LD50-values. These data were chosen because they cannot be supplied by otehr means, in contrast to minimal skin and eye toxicity, which may be tested in human volunteers. No other ways of selection were used. The list consists of a majority of pharmaceuticals, but include solvents, pesticides and metals, as well.

The study is not made with coded substances, because this wold be so costly and circum antial as to inhibit the realisation of the program. Most testing is blind, in the sense that a majority of testers submit their results for the successive segments of the list, before evaluation is made and human reference data are published. Not blindfolded results are also welcome to be evaluated, but it will always be stated in publications if a ceratin set of data was submitted blind or not.

Participants have to buy the substances themselves. No distribution of chemicals has been arranged. This will include that the program will be performed with different batches of chemicals, in some cases not of the highest purity. We consider this a minor inaccuracy. First, the study is among the first programmes of its kind-it is better to achieve a rough and relatively broad and fast charting of the field, that loose momentum, due to a prematue concern about the best possible

precision. Second, the human toxicity compared against have not data for high-purity or batched chemicals. Third, only a synchronous study of limited duration may benefit from the same batch of chemicals. MEIC is going on for many years, so the same batches would be of very different age (activity) with time.

Participants ought to submit dose-response curves (if applicable) with values in mM for IC50 an IC10 (50% and 10% inhib. conc.). Since the evaluation will be a quantitative, linear, point-to-point procedure, no cut-off results are permitted (or, at least, they cannot contribute to validation). A few values may be missing (one or maximum two per ten values) without disturbing evaluation. There are no central directives about suitable solvents. Solvents are considered as a part of the method used. When in doubt, participants may use two different solvents or dissolution measures.

Development of new methods

Today we have an over-veiw of most methods in the project. If a known standard method is not performed, we actively try to persuade a laboratory to contribute with this specific method. We also try to encourage MEIC participants to develop quite new methods, the need for which is suggested by the evaluation process. To make complete models of human general toxicity it is obvious that many new methods ought to be developed, such as long term cytotoxicity tests (7), stimulatory toxicity, reversibility of toxicity, a row of toxicokinetic tests, etc. The biochemical cytotoxicity assays also ought to be supplemented by morphology studies of cells.

Steps of evaluation

Several types of toxicity will be evaluated. The order of priority is acute lethal toxicity, acute sublethal toxicity, skin irritancy, chronic systemic toxicity, target organ toxicity, etc. Within each group, evaluation starts by establishing the evaluation methods with use of available results from the first ten reference chemicals. If no serious objections are rised, these models are then used to evaluate the rest of the chemicals. The timing of evaluation is determined by the effectivity of participants-when a majority have tested a segment of the list, e.g. nos. 11–30, evaluation is made.

Evaluation methods

At the start of the program these methods were outlined (1). The description is till valied. To summarize, a few techniques were going to be used, i.e. linear regression with predetermined analysis of outliers, cytotoxic quotients to determine the cytotoxicity of blood and tissue concentrations, and, finally, multivariate PLS-analysis (partial least squares) (8, 9). Each type of toxicity is validated in two ways, used in parallel, The first way is hard modelling (biased validation) by which the accuracy of prediction of supposed effective combinations of in vitro data is evaluated. The other way is soft modelling, when all *in vitro* data are statistically combined to models with the best fit to in vivo data. The PLS-technique can be applied to both the hard and the soft modelling, but is the method of choice in soft modelling (8, 9).

At the start of the programme, three steps of evaluation were described: 1. Comparison of animal data with human data, 2. Comparisons of *in vitro* data and human, toxic, blood and tissue concentrations, and 3. Comparisons of *in vitro* data and human toxic dosage (1). In the two last steps, and especially in the third step, the *in vitro* toxicity data should be combined with *in vitro* toxicokinetic data-that is, the in vitro toxicity data must be transformed with help of a toxicokinetic adapter to be able to predict dosage.

The direct modelling of *in vivo* toxic doage entirely by in vitro data once outlined (1), was over-optimistic. There are simply not enough *in vitro* toxicokinetic tests to permit this. Therefore, the last step has been modified (10, 11) to an adaption of *in vitro* toxic concentrations for prediction of dosage, with use of *human* toxicokinetic data for the chemicals. A late phase of MEIC may try to

model toxic dosage exclusively by *in vitro* data, however.

Preliminary evaluation

At present, thirty laboratories have submitted results from testing of the first ten MEIC chemicals by altogether 100 methods. These results have been used to demonstrate validation models for acute lethal toxicity (9–11) and acute, sublethal, mild/moderate toxicity (12, 13). As a third type of preliminary validation, in vitro/in vitro comparisons have been performed with the successively enlarging data base (9, 14, 15, 16), the results of which has been or will be presented at the 1990, 1991, and 1992 U.S. Tissue culture Association conferences.

The results from te *in vitro/in vivo* comparisons of acute toxicity (9–13) for the first ten MEIC chemicals have been very encouraging, i.e. most *in vitro* cytotoxicity tests were predicting human acutely toxic concentrations and dosage (the latter with help of simple human toxicokinetic data) better that the prediction of acute human toxicity by rodent LD50 data. The results from the in vitro/in vitro comparisons were also positive for the development of an *in vitro* toxicity testing. First, human cells seemed to predict human toxicity better than animal-derived cells, thus overcoming the species-gap in animal testing. Second, organ-specific cells and functions could in some cases be used to predict human target organ toxicity, by differential cytotoxicity analysis, i.e. by comparing basal cytotoxicity results with results from organ-specific cells (16, 17). Third, most basal cytotoxicity tests with cell lines and use of exposure periods o 24–72h gave similar results, irrespective of the cell type and the toxicity measurements used. This is probably due to a cloe integration of the basal cell functions, spreading primary chemical effects on specific organelles/functions to all organelles after 24h. This will facilitate futur testing of basal cytotoxicity-only one or a few systems will suffice to get the appropriate information.

In the near future, preliminary evaluation will demonstrate and establish models for skin irritancy and chronic toxicity.

Independent MEIC publications

As many as 17 manuscripts, including 7 published articles (listed in MEIC Newsletter Vol. 3, No. 4, 1992) have already been written independently by MEIC participants, presenting and analysing results from the laboratory's own MEIC tests. Many of these papers perform evaluation especiallyu of the *in vitro/ in vitro* type. Such spontaneous, independent evaluation is a very important supplement to the MEIC-committee validation efforts.

Prospects of MEIC

MEIC may be an effective approach to sort out and design batteries of *in vitro* tests which can be used to supplement and replace animal tests for certain types of general toxicity. In that way MEIC may contribute to a more ethical, and maybe, more economical future toxicity testing.

However, hte MEIC programme may result in findings which will be considered still more important than making toxicity tests more ethical. MEIC may in fact improve toxicological science, by establshing a st of more effective elementary concepts.

Many other sciences have developed from compilations of anecdotic and epiphenomenal knowledge to a structured, systematic understanding, through a recognition of the interplay of key elements. The key elements of chemistry are obviously the atoms and molecules. In medicine, the key elements were recognized in 1858, when Rudolf Virchow introduced a pathology based on cells and the reactions of cells. Toxicology is still lacking suitable key elements, permitting a rational way of synthesizing and thus understanding various phenomena. The unit for toxic reactions use today, i.e. the laboratory animal, is, bty far, too complicated to be a good elementary unit. A much better such unit wold be the cell, and especially the basal cell functions, in similarity to patholgy and medicine. The three assumptions of the MEIC programme are an outline of a more effective

and target-related toxicological science. If these hypotheses could be verified by the participants of MEIC, toxicology nad toxicity testing might develop to a more rational procedure, able to cope with the ever increasing demands of securing human safety in a world full of poorly tested chemicals (18).

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