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# REVIEW ON *IN VITRO* TESTS FOR PREDICTING DRUG – DRUG INTERACTIONS

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**ABSTRACT**
Prediction of drug-drug interactions from in vitro studies have become rapidly expanding in field of research. Over the last 20–30 years our understanding of drug metabolism in man has greatly increased to accumulate knowledge and to the use of in vitro and even in silico models. Several In vitro & In silico models are proposed for assessing drug interactions. Although the models can easily predict the type, mechanism, and even magnitude of interactions, they often fail to predict the clinical consequences. The main goal of these tests is to eliminate as early as possible those molecules which would fail during development, so as to develop only those products which are reasonably likely to succeed. Ideally, sufficient information on potential drug interactions should be obtained before a compound is selected for development. In this context, information from in vitro inhibition studies has become important in choosing new candidate drugs for development.

**KEYWORDS**: In vitro, In silico, in silico prediction drug-drug interactions, drug-metabolising enzymes, inhibition, induction, recombinant enzymes, CYP enzymes, validation, coca-2 cells, immuno chemical probes, FDA, EMEA.

## INTRODUCTION

### A) *IN VITRO* METHODS

*In vitro* methods means using cells and tissues outside the body in an artificial environment, which are used to determine the safety or effectiveness of a drug or ingredient.

### B) BASIC CONSIDERATIONS

***i) Altered absorption:*** Drug absorption is altered by chelation or complex formation (e.g. the absorption rate of acetaminophen is increased by metoclopramide and delayed by propantheline) (Fleisher, D. *et al. 1999)*

***ii) Altered distribution:*** Competition of two drugs for the same plasma protein-binding sites can result in an increased free plasma concentration of the lower affinity drug. (Kedderis, 1997)

***iii) Induction:*** Induction or increased synthesis of one or more drug-metabolizing enzymes leads to enhanced metabolism and hepatic clearance of all substrates for those particular pathways.(Fleisher, D. et al 1999)

***iv) Inhibition:*** Inhibition or decreased metabolism can result from competition between drugs for the enzyme's binding sites. (Lin, J.H., Lu 1998)

***v) Altered excretion:*** Competition for renal anion or cation transport systems changes in urinary pH (e.g. sodium bicarbonate increases renal elimination of phenobarbital) and inhibition of renal metabolism (Bonate, P.L. et al 1998)

## DRUG-DRUG INTERACTIONS

- According to certain sources, drug-drug interactions could be responsible for a hundred thousand deaths each year among patients. This, however, is very hard to objectivate, since official death statistics seldom identify and clearly document fatal outcomes that may be related to drug interactions. (Lazarou *et al.* 1998; Ingelman-Sundberg 2001).
- Drug interactions may be caused due to administration of another compound, another drug, an environmental pollutant (i.e. cigarette smoke, polycyclic hydrocarbons, solvents …), or an ingredient or additive present in the diet (cruciferous vegetables, grapefruit juice, sweeteners, colourings …).
- As more drugs become available and are used concomitantly, the potential for drug interactions increases. Recently, newly marketed drugs have been withdrawn from the market because of unacceptable interaction profile. (Reuters Medical News 2001).
- Understanding and anticipating drug interactions is a necessary part of rational therapeutics. The clinical

importance of any drug interaction depends on several factors, including the condition of the patient, the drugs administered, the route of administration, the environment, the therapeutic index, the timing of administration of two or several drugs etc. This leads to considerable inter individual variations.

**a) Determination of drug-drug interactions**

- *In vitro* systems have become widely used as screening tools and for the study of the mechanisms of drug-drug interactions. (Fuhr *et al.* 1996).
- The availability of human tissues and recombinant human CYP enzymes has greatly contributed to the development of *in vitro* screening tools for predicting potential *in vivo* drug interactions.
- Since drug interactions are normally considered undesirable in drugs under development, their occurrence must be documented much earlier in the development process, if possible during the selection phase (Lin 1998).

**b) Possible causes of drug-drug interactions**

• Competition for GI absorption
• Interaction during membrane crossing (blood vessels, hepatic, renal)
• Binding to plasma proteins
• Binding to transport proteins and p-glycoproteins
• Pharmacodynamic interactions at receptor level
• Inhibition& inhibition of metabolism
• Competition for active renal excretion

**c) DRUG-DRUG INTERACTIONS MAY PRODUCE UNDESIRABLE AND SOMETIMES HARMFUL EFFECTS**

- ADR results most often from drug abuse, misuse, over prescription, bad prescriptions, wrong co medication, and genetic factors, but also from effects produced by alimentary products, environmental contaminants, etc(Thummel,.1998)
- According to Heerey et al., 7% of acute hospital admissions in Ireland result from ADR, of which 25% are due to drug.drug interactions (hereafter called DDI).
- Most of these statin-linked ADRs are due to their co prescription with other lipid-lowering agents and are thus the consequence of DDI.

**Some Examples of Drug-Drug Interactions and Their Consequences**

- Terfenadine and grapefruit juice may produce cardiac arythmia. (Thummel, 1998)
- Rifampicine and contraceptives may produce contraception failure. (Capone *et al* 1996)
- Ketoconazole and cyclosporine, may lead to toxic effects.

**INVITRO DRUG METABOLISM**

- FDA and EMA have suggested a programme of in vitro studies (using human liver microsomes and hepatocytes) to be conducted for compounds under development to assess their potential effects on CYP450 enzymes and major drug transporters (e.g. P-gp).
- The results of these in vitro studies are then used to assess whether a clinical assessment in vivo for potential drug-drug interaction liability is required and ultimately to direct product safety labelling.
- Importantly, a negative result to a properly conducted in vitro interaction assessment study is accepted by Regulatory Authorities, and confirmation in vivo is generally not required.

***In vitro studies can therefore contribute greatly to reducing development time and cost***

**a) Drug-drug interactions due to induction of metabolism**

✓ Enzyme induction most often results in a reduced pharmacological effect due to increased drug metabolism. Rifampicin, one of the most potent CYP-enzyme inducers in man, is known to accelerate the metabolism of ethinyl-estradiol, leading to contraceptive failures.

**b) Drug-drug interactions due to inhibition of metabolism**

Inhibition of P450-supported metabolism can result from different mechanisms:

**1) REVERSIBLE INHIBITION**

**i) Competitive inhibition:**

- Substrate and inhibitors compete for the same binding site: the active site of the enzyme.

**ii) Non-competitive inhibition**

- Substrate and inhibitor bind to different sites.
- Binding of the inhibitor results in conformational changes resulting in a reduced metabolic rate.

**2) IRREVERSIBLE INHIBITION**

- Irreversible inhibition occurs when a drug is oxidised to an intermediate that coordinates so tightly to the prosthetic haem that it can only be displaced under special experimental conditions.

Example: Mechanism-based inactivation of CYP3A4 by macrolide antibiotics.

**Table 1: Drug Related Side Effecs.**

| Drug | Side Effect |
|---|---|
| Terfenadine, Astemizole, Pimozide, Cisapride | Ventricular arrythmia |
| Some statins | Rhabdomyolysis |
| Midazolam, benzodiazepine | Increased sedation |
| Sildenafil, phosphodiesterase inhibitors | Hypotension |
| Carbamazepine | Ataxia |
| HIV protease inhibitors | Increased bioavailability |
| Angiotensin converting enzyme inhibitors | Reduced blood pressure |
| | (Dresser *et al* 2000,Goho2001) |

**EXPERIMENTAL APPROACH USED IN *IN VITRO* TESTING TO PREDICT DRUG -DRUG INTERACTIONS**

***a) Sub cellular Fractions of Human Liver Tissue***
***b) Whole cell models***
***c) Caco-2 cells monolayer's***
***d) Immunochemical probes***

***a) Sub cellular Fractions of Human Liver Tissue***

- New drug effects are observed on human liver microsomes by CYP pathways & collected from multiple donors.
- Centrifugation of homogenized liver gives hepatic microsomes (sub cellular tissue).
- Microsomal fraction include the cytochrome P450 superfamily, the flavin-containing mono-oxygenases, epoxide hydrolases, and a variety of transferases (e.g. the UDP-glucuronosyltransferases).
- Microsomal preparations require the addition of exogenous cofactors, including a source of NADPH.
- Predictions of drug interactions from cell-free systems such as microsomes may be irrelevant if marked in vivo differences between plasma concentrations and intracellular hepatocyte concentrations.

# Advantages of the system include ease of preparation, commercial availability, and longterm stability during cryopreservation.

***b) Whole Cell Models***

- Isolated hepatocytes in suspension or primary culture and precision-cut liver slices offer numerous advantages over subcellular fractions, including a full complement of hepatic drug- metabolizing enzymes, endogenous cofactors.
- The characteristics may vary depending on age, health, genotypic status of donor, diet, alcohol e.t.c.
- Short-term stability of enzymatic activities represents the major problem with hepatocytes and liver slices
  <3-4 h for suspensions.
  <24 h for cultures or slices.

# Advantages of preserving the tissue cytoarchitecture and cell- tocell communications.

***c) Caco-2 cell monolayers***

- Caco-2 cells are derived from human colon cancer cells. When cultured on porous membranes, they differentiate spontaneously into monolayers of polarized cells. (T.F. Woolf ed., 1999)
- At the level of the human intestine they serve as a surrogate model for absorption and metabolism.

**ADVANTAGES OF Caco-2 CELLS**

- Caco-2 cell monolayers are a useful system in which to investigate drug interactions resulting from inhibition of the P-glycoprotein efflux mechanism.

**DISADVANTAGES of Caco-2 cell monolayers**

- Include the under expression of metabolizing enzymes and a time-dependent loss of enzyme activity in culture. (T.F. Woolf ed., 1999)

***d) Immunochemical Probes***

- Selective inhibition of certain CYP enzymes in microsome preparations can be achieved through use of polyclonal or monoclonal antibodies.

**Disadvantages**

- Lack of wide commercial availability of these antibodies.
- Deficient antibody selectivity between subfamily members.
- Inability to achieve 100% inhibition (due to large size of the antibody molecule does not permit access to all isoform molecules).
- High degree of inter-laboratory variations.

***IN SILICO* PREDICTION OF DRUG INTERACTIONS**

- SAR and QSAR studies, elucidation of the three-dimensional structure of proteins, receptors, enzymes, etc., will soon allow an objective representation of the binding of a drug to its biochemical target.
- HTS (high throughput screening), allows correlation studies between *in vitro* and *in vivo* data. (Ter laak,*et al* 2001)
- New software applications are being developed to handle these data and to extrapolate the results to similar situations or products.
- Dynamic computer-based method, called Quantitative Drug Interactions Prediction System (Q-DIPS), has been developed to make both qualitative deductions and quantitative predictions on the basis of databases containing updated

information on CYP substrates, inhibitors, inducers, and pharmacokinetic parameters.

***NECESSITY OF A VALIDATION PROCESS***

- ✓ ECVAM, the European Centre for Validation of Alternative Methods, has undertaken to organise scientific meetings and coordinated studies to promote progress toward validation of metabolic studies.
- ✓ Validation of *in vitro* tests takes the form of multi-study trials. These consist of several separate studies conducted and reported without knowledge of the test item randomisation code (Cooper-Hannan *et al.* 1999).
- ✓ Although more and more non-academic laboratories are conducting their *in vitro* studies in compliance with good laboratory practice rules and thus offer some guarantee concerning the traceability of the generated data, major improvements are still necessary.

**EXTRAPOLATION TO THE IN VIVO CLINICAL SITUATION**

- ➢ Extrapolation of *in vitro* results from kinetic studies necessitates the defining of several parameters in order to evaluate the concentrations of both substrate and inhibitor.
- ➢ Extrapolating *in vitro* results to the *in vivo* situation in order to predict clinical outcomes remains a very hazardous task.
- ➢ Large studies correlating *in vitro* with *in vivo* data obtained on the same molecules will certainly be particularly helpful in specifying, adjusting, and understanding the numerous parameters involved in the process.

**DISCUSSION**

***(Is it possible to answer clearly the question: Are DDI predictable?)***

- The tools in our hands today certainly allow us to study the phenomenon correctly and, in a large number of cases, to predict correctly, at least on a qualitative basis, DDI from simple *in vitro* studies.
- Moreover, as recommended by the regulatory agencies (FDA, EMEA), this should be done for all new drugs under development.
- To avoid DDI, it is desirable to develop drugs that are neither potent CYP inducers nor inhibitors, the metabolism of which is not affected by other common drugs.

**CONCLUSION**

- Efforts are still needed in order to improve the quality, reliability, and reproducibility of *in vitro* assays in order to reduce interlaboratory variations due to the quality of the biological material used, the incubation conditions, and the analytical techniques used to quantify the reaction products.
- Validation of methodologies will by no means resolve all the problems linked to drug-drug interactions prediction, but will necessarily contribute greatly to safer extrapolations. This is in itself a sufficient justification for further studies in this direction.
- *In vitro* prediction of drug-drug interactions help the pharma market to manufacture the safe product.
- Aspects such as protein binding and transporter-mediated drug-drug interactions (not discussed in this paper) are the focus of many ongoing studies. These will certainly shed new light on the subject and help to specify the extrapolation parameters.

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