

IN VITRO MODELS IN THE PREDICTION OF METABOLISM AND TARGET ORGAN SPECIFICITY

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Introduction

Metabolism can result in bioactivation rather than in detoxification of xenobiotics. The purpose of this work was to compare the cytotoxicity of selected compounds in metabolically fully competent (primary culture rat hepatocytes) vs. metabolically non-competent (HepG2 and Balb/c 3T3 lines) cells.

Methods

Cultured media:

HepG2 cell: Dulbecco's modification of Eagle's medium + 10 % fetal calf serum, 4 mM glutamine, 100 IU/ml penicilin and 100 mg/ml streptomycin.

Balb/c 3T3: Dulbecco's modification of Eagle's medium + 10 % new born calf serum, 4 mM glutamine, 100 IU/ml penicilin and 100 mg/ml streptomycin.

Rat hepatocytes: Williams E medium + 10 % new born calf serum, 2 mM glutamine, 0.2 % bovine serum albumin, 0.1 mM insulin, 100 IU/ml penicilin and 100 mg/ml streptomycin.

Cell culture:

HepG2 and **Balb/c 3T3** cell lines were purchased from The European Collection of Cells Cultures (ECACC). Cells were thawed, resuspended in culture medium and cultured (37 °C, 90 % humidity, 5 % CO₂) in tissue-culture flask. Cells were removed from the flask by trypsinization, resuspended in culture medium and sub-cultured into other flasks. Cells were counted by trypan blue exclusion method in Bürker chamber.

Rat hepatocytes were isolated from male rats (Sprague & Dawley) by enzymatic perfusion. Animals were anaesthetized, their vena porta was cannulated after laparotomy and liver were perfused by Hank's solution and given to collagenase solution for disintegration. Obtained cell suspension was filtered through sterile gauze and centrifugated (at 60 g). Cells were counted by trypan blue exclusion method in Bürker chamber.

The ECOD activity - quality control criterium for metabolic competence:

Suspension of hepatocytes in culture medium was seeded on collagen type I coated Petri dishes (100 mm I.D.) at density of 80000 viable cells per cm². The ECOD activity (activity of 7-ethoxycoumarin O-deethylase), representative of several CYP450 activities, was measured in each hepatocyte culture before the toxicity screening. Cells were shifted to serum free culture medium (1mM dexamethasone instead of serum) after 1-4 hours. Hepatocytes were cultured as monolayer (37 °C, 90 % humidity, 5 % CO₂).

MTT test for basal toxicity: Cells were seeded in 96 well plates (for hepatocytes collagen-coated) in a density of 25000 (hepatocytes), 60000 (HepG2) or 2500 (Balb/c 3T3) per well (100 ml per well). All cells were seeded in culture medium. Compounds were dissolved in serum free medium directly or after dissolution in DMSO or ethanol. Solutions of chemicals were added to 96 well plates (100 ml per well). The cytotoxicity was assessed after 24-hour incubation using MTT assay, based on the ability of viable cells to reduce the yellow tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) to blue insoluble formazan, in 96 well plates. Sodium lauryl sulfate was used as a positive control.

Absorbance of resulting colored solution was measured in microtiter plate reader at 550 nm. Calculation of cell viability expressed as absorbance was made for each concentration of the test chemical by the mean of the six replicate values per tested concentration (blank was subtracted). **Results are expressed as means from 3 independent experiments (at least).**

Results

| COMPOUND /cell line | HepG2 | Rat hepatocytes | Balb/c 3T3 |
|--------------------------|-------------------|---------------------|-------------------|
| | IC50 (mol/l) | | |
| sodium lauryl sulfate | 1.7E-04 * | 2.9E-04 * | 1.4E-04 |
| atropine sulfate | 3.2E-03 * | 5.3E-04 * | 5.3E-04 |
| pentachlorophenol | 1.1E-04 | 9.0E-05 * | 8.4E-05 |
| verapamil | 2.3E-04 | 7.7E-05 * | 1.1E-04 |
| malathion | non toxic * | non toxic * | 2.1E-04 * |
| orphenadrine | 3.5E-04 * | 8.7E-05 | 1.2E-04 * |
| rifampicine | 5.0E-04 * | 5.5E-04 * | 3.7E-04 * |
| amiodarone HCL | 2.4E-05 | 2.6E-05 * | 2.7E-05 * |
| colchicine | > 1.0E-03 | > 1.0E-03 * | > 1.0E-03 * |
| valproate | 4.6E-02 * | > 1.0E-03 * | > 1.0E-03 * |
| acetylsalicylic acid | > 1.0E-03 * | > 1.0E-03 * | > 1.0E-03 * |
| 2-propanol | > 1.0E-03 * | > 1.0E-03 * | > 1.0E-03 * |
| acetaminophen | > 1.0E-03 * | > 1.0E-03 * | > 1.0E-03 * |
| caffeine | > 1.0E-03 | > 1.0E-03 * | > 1.0E-03 * |
| tetracycline HCl | > 1.0E-03 * | 5.4E-04 * | > 1.0E-03 * |
| carbamazepine | non toxic * | non toxic * | non toxic * |
| digoxin | 4.8E-06 | > 1.0E-03 * | non toxic * |
| 17alpha-ethynylestradiol | 1.0E-04 * | 1.1E-04 * | large fluctuation |
| cycloheximide | large fluctuation | large fluctuation * | large fluctuation |
| cyclosporine A | non toxic * | 9E-06 * | large fluctuation |
| mercury(II) chloride | tested | tested | large fluctuation |
| diazepam | tested | tested | tested |
| phenobarbital | tested | tested | tested |

Results marked by asterisk do not pass acceptance criterium 3; * **Saturated solution is not toxic (viability > 50%)**
Reaction with medium?

Discussion

Topics to be discussed: acceptance criteria and other problems (e.g. reaction of the compound with medium, saturated concentration of the compound, large fluctuation etc.)

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