Toxicology testing using upcyte® hepatocytes

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INTRODUCTION

Summary and novelty: We have developed a technique which causes primary human hepatocytes to proliferate whilst retaining an adult phenotype. The resulting "upcyte® hepatocytes" have the capability to proliferate and express sufficient drug metabolizing activities, a combination which makes them unique. So far 5 different donors of upcyte® hepatocytes have been generated (#10, #740, #151, #653, #422).

Cytotoxicity assay: The cytotoxicity of 31 compounds was measured using ATP and LDH content and MTS metabolism in upcyte® hepatocytes from four donors. The cytotoxicity of the majority of compounds was donor-dependent Donor 653 was generally less susceptible to cytotoxicity than donors 422A, 151 and 10. There was a good intra- and inter-experimental reproducibility and the predictive capacity of the assay was good such that known non-hepatotoxicants were clearly negative and compounds that were associated with hepatotoxicity caused cytotoxicity in upcyte® hepatocytes.

Micronucleus assay: We optimized the assay conditions incorporating upcyte® hepatocytes into the micronucleus test. A treatment duration of 96 h was optimal for detecting the genotoxicity of the direct-acting, mitomycin C, and the bioactivated compound, cyclophosphamide, whilst negative and "false“ positive compounds were correctly identified as negative. The basal MN rate of upcyte® hepatocytes was affected by pre-culture period and medium components. The% MN in control and genotoxicant-treated upcyte® hepatocytes was similar at different growth stages.

Cytotoxicity testing using upcyte® hepatocytes

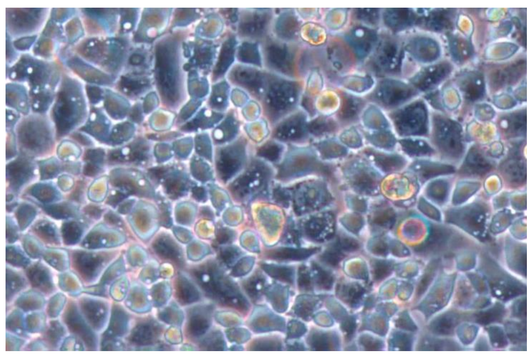

Figure 1: Morphology of confluent upcyte® hepatocytes showing well-defined cuboidal shape characteristic of primary hepatocytes

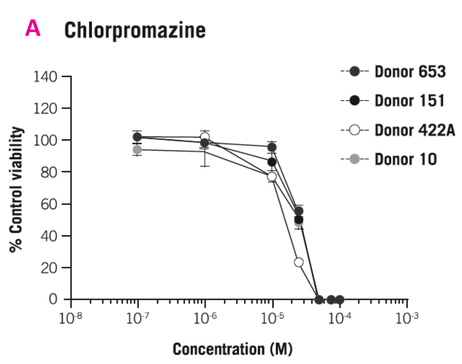

Figure 2A: Cytotoxicity of chlorpromazine in upcyte® hepatocytes from different donors (using MTS as the viability measurement)

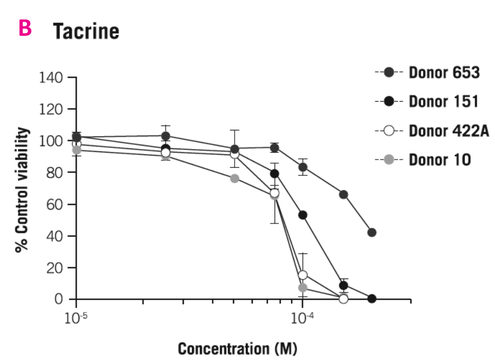

Figure 2B: Cytotoxicity of tacrine in upcyte® hepatocytes from different donors (using MTS as the viability measurement)

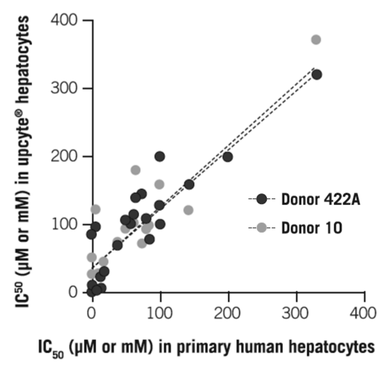

Figure 3: A comparison of the IC_{50} values generated in upcyte® hepatocytes from donors 422A and 10 with those cited in the literature for primary hepatocytes. Values are from 31 compounds using MTS metabolism.

For cytotoxicity screening, the cells were pre-cultured in 96-well plates as 20 monolayers for 3 days and then treated with test compounds for 4 days. The viability was measured using ATP and LDH content and MTS metabolism in upcyte® hepatocytes from four donors. For some compounds, such as chlorpromazine, the cytotoxicity was similar in upcyte® hepatocytes from different donors (Figure 2A). For other compounds, such as tacrine, the cytotoxicity was doner-dependent e.g. Donor 653 was generally less susceptible to cytotoxicity than donors 151, 422A and 10 (Figure 2B).

There was a good intra- and inter-experimental reproducibility and the predictive capacity of the assay was good such that known non-hepatotoxicants were clearly negative and compounds that were associated with hepatotoxicity caused cytotoxicity in upcyte® hepatocytes. Moreover, there was a good correlation between the MTS IC_{50} values from our studies with those obtained from the literature for the same compounds in primary human hepatocytes (using MTT), supporting the use of upcyte® hepatocytes as an alternative model to primary cells (Figure 3).

Genotoxicity testing using upcyte® hepatocytes

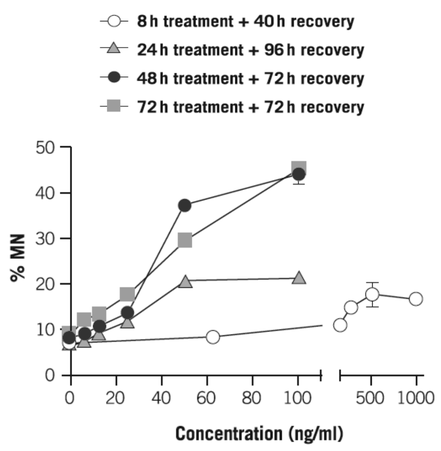

Figure 4: Effect of treatment and recovery duration on the % MN of cells treated with MMC

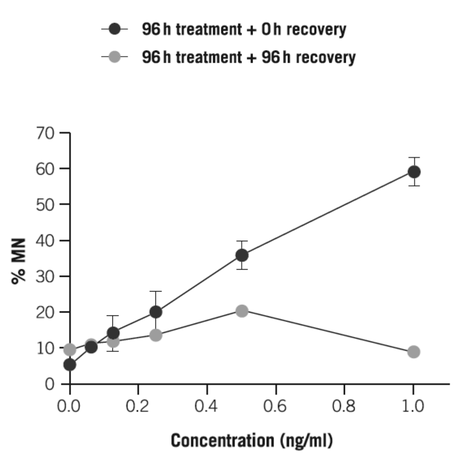

Figure 5: Effect of a 96h recovery on the % MN in cells treated with etoposide

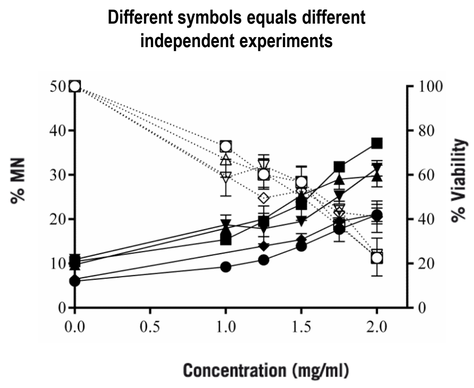

Figure 6: Reproducibility of the % MN and cytotoxicity in upcyte® hepatocytes treated with cyclophosphamide

Chemical (CAS number)	Outcome from *in vitro* MN assay
True positives	
Mitomycin C (MMC) (50-07-7)	Positive in 5 out of 5 experiments
Etoposide (33419-42-0)	Positive in 3 out of 3 experiments
Taxol (33069-62-4)	Positive in 3 out of 3 experiments
Cyclophosphamide (6055-19-2)	Positive in 6 out of 6 experiments
Benzo[a]pyrene (50-32-8)	Positive in 4 out of 4 experiments
True negative chemicals	
Ampicillin tryihydrate (7177-48-2)	Negative in 4 out of 4 experiments
Cyclohexanone (108-94-1)	Negative in 5 out of 5 experiments
Melamine (108-78-1)	Negative in 5 out of 5 experiments
Tris(2-ethylhexyl)phosphate (78-42-2)	Negative in 3 out of 3 experiments
False positive chemicals	
2,4-dichlorophenol (120-83-2)	Negative in 3 out of 3 experiments
Benzyl alcohol (100-51-6)	Negative in 4 out of 4 experiments
Curcumin (458-37-7)	Negative in 6 out of 6 experiments
Urea (57-13-6)	Negative in 4 out of 4 experiments
Sulfisoxazole (127-69-5)	Negative in 3 out of 3 experiments
Sodium saccharin (128-44-9)	Negative in 3 out of 3 experiments

Table 1: Outcome of the testing of true positive, true negative and false positive chemicals

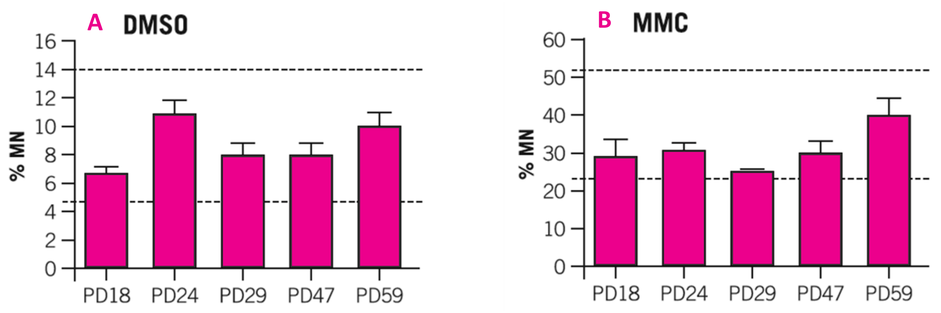

Figure 7: Effect of growth stage (PD) on % MN in cells treated with DMSO and MMC

The dotted lines represent the highest and lowest values measured for DMSO (highest = 14.1%, lowest = 3.7%) and MMC (highest = 52.2 %, lowest = 23.3 %) in upcyte® hepatocytes from Donor 740 at a PD of 25. Values are mean ± SD of triplicate wells in one experiment.

Optimization of treatment and culture conditions for the in vitro MN test

Different treatment (8 to 96 h) and recovery durations (0 to 96 h) were tested to determine the optimal conditions for detecting genotoxicants in upcyte® hepatocytes. Longer treatments resulted in higher formation of MN and lower concentrations of test compound were needed to cause the same extent of MN formation (Figure 4). Figure 5 shows that after a 96 h treatment, the cytotoxicity of etoposide was higher when a subsequent recovery period (without test compound) was included. For this reason, a treatment duration of 96 h, without a recovery period was selected as optimal for all compounds. Under these conditions, upcyte® hepatocytes can be incorporated into the in vitro MN test to detect both directly acting (e.g. mitomycin C, etoposide (Figure 5)) and metabolically activated genotoxins (e. g. benzo[a]pyrene, cyclophosphamide), whilst true negative and "false" or "misleading" positive compounds were reproducibly and correctly identified as negative (Table 1). The basal MN rate of upcyte® hepatocytes from three other donors was higher than that in Donor 740 (28% compared to -7%, respectively); therefore, the medium was modified by adding oncostatin M and EGF to decrease inherent MN formation (data not shown). There was a very good reproducibility between experiments with respect to the % MN formed and the cytootoxicity in upcyte hepatocytes (Figure 6).
The %MN in control (DMSO) and genotoxicant-treated upcyte® hepatocytes was similar at different growth stages and were within the inter-experimental variation values measured for cells at a population doubling (PD) of 24 (Figure 7)

CONCLUSION

In conclusion, these data support the use of upcyte® hepatocytes in the MN test, especially since these cells combine proliferation with a metabolic capacity- thus negating the need for an exogenous bioactivation system. Our data also show that upcyte® hepatocytes can be used as a suitable alternative to primary human hepatocytes for hepatotoxicity screening- combining predictivity and reproducibility with a substantial cell source