Fresh vs Cryopreserved Hepatocytes in Evaluating Human-Specific Effects of Dioxin

Aruna Koganti[1], Neil S Jensen[1], Jay B Silkworth[2], Paul M Silber[1]

[1]In Vitro Technologies
[2]GE, Global Research Center

Introduction

The potency and toxic effects of dioxins in humans have been traditionally extrapolated from experiments in laboratory animals[1]. Studies indicate that this approach may overestimate the toxic effects of dioxins in humans[2]. Comparison of the effects of these chemicals in cultured hepatocytes from humans and laboratory animals may provide relevant scaling factors that make the extrapolation from animal experiments more accurate[3]. However, the limited availability of freshly isolated human hepatocytes poses a major drawback to the use of this experimental system. The availability of cryopreserved human hepatocytes that can be cultured may circumvent this problem. This study evaluated the induction of cytochrome P4501A (CYP1A) enzyme activity by dioxin in freshly isolated and cryopreserved human hepatocytes.

It has been established that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxin-like compounds exert their biological effects through the aryl hydrocarbon receptor (AhR)[4]. The ligand-activated AhR enhances transcription of a battery of genes. A number of genes encoding xenobiotic-metabolizing enzymes, such as CYP1A1, CYP1A2, CYP1B1, glutathione S-transferase, and UDP-glucuronosyltransferase are members of the AhR target gene battery. Induction of these enzymes is a common response to dioxins observed across species, and it can be easily quantified. Studies have also shown that induction of CYP1A2 leads to hepatic sequestration of TCDD in rodents[5], elevating the potential for liver to be a target organ. Because, cultured hepatocytes have been widely used in evaluating the induction potential of many xenobiotics, the use of cryopreserved human hepatocytes may provide a reliable mechanism-based approach for evaluating relative species potencies for the dioxins.

Materials and Methods

Hepatocytes used in these experiments were isolated from non-transplantable human liver tissue and cryopreserved based on the methods of Li, et.al.[6,7]. The liver tissue was obtained using an IRB-approved protocol. Hepatocytes were isolated fresh or were obtained from the cryopreserved hepatocyte bank at In Vitro Technologies, Inc., and cultured as collagen sandwich cultures. Representative micrographs of the cultures are shown in Figure 1. After cultures were established, the cells were treated with TCDD for 48 hours. Following treatment, the medium containing TCDD was removed and the cells were treated with ethoxyresorufin, a specific substrate for CYP1A. The formation of resorufin from ethoxyresorufin was quantified using a fluorescent plate reader assay to determine CYP1A enzyme activity. TCDD was evaluated at each of 11 concentrations in freshly isolated hepatocytes from 5 human donors, and plateable cryopreserved hepatocytes from another 5 human donors.

Results and Discussion

The data show that complete concentration-response relationships were obtained for TCDD in both freshly isolated and cryopreserved hepatocytes from all donors (Figures 2 and 3). These relationships were similar in freshly isolated or plateable cryopreserved hepatocytes in that the variability between donors and the ranges of EC_{50} (TCDD concentration where half of the maximal response is observed) values calculated were comparable.

The inter-donor variability was estimated based on the TCDD concentration where the response above background levels was first observed for each donor. The inter-donor variability for responding donors ranged from 0.01 nM to 0.1 nM and 0.01 nM to 0.3 nM for fresh and cryopreserved hepatocytes, respectively (Figures 2 and 3). The EC_{50} values were calculated by fitting the data to a sigmoidal variable-slope curve (Table 1; GraphPad Prism, version 4.0). The EC_{50} values for fresh hepatocytes ranged from 0.0455 nM – 1.41 nM and from 0.122 nM – 1.33 nM for

cryopreserved hepatocytes. The maximal responses obtained with cryopreserved hepatocytes appeared to be 10-fold lower compared to freshly isolated hepatocytes. The reason for this is not known at this time.

The data presented demonstrate the utility of the cryopreserved hepatocyte model in establishing human-specific concentration-response relationships and in evaluating inter-individual variability. *In vitro* experiments with cultured hepatocytes facilitate evaluation of multiple parameters such as concentration-response relationships for a given chemical or complex mixture, relative potencies of these chemicals, species differences, and inter-individual differences in humans[8]. In addition, the concentration-response obtained in the *in vitro* test system may help to elucidate the correlation between the response and the AhR binding affinities of the various ligands. Evaluation of individual chemicals, in comparison to mixtures, may also help elucidate synergistic, additive, or inhibitory effects with complex mixtures. When hepatocytes from multiple species are used, this model could potentially provide relevant scaling factors[8]. Other advantages of this *in vitro* model are that it requires very small quantities of chemicals, thus limiting exposure of personnel conducting the experiments, and it does not involve large numbers of animals. In addition, valuable information can be obtained at a relatively low cost. Cryopreserved hepatocytes provide the added advantages of ease of scheduling and a test system that is available for repeated experiments over time using cells from the same donor(s).

Conclusions

Freshly isolated and cryopreserved hepatocytes produced similar results in the evaluation of inter-donor variability and EC_{50} value estimations. Thus, it is believed that cultured cryopreserved human hepatocytes represent a suitable test system to evaluate human-specific effects of dioxins or other similar chemicals.

References

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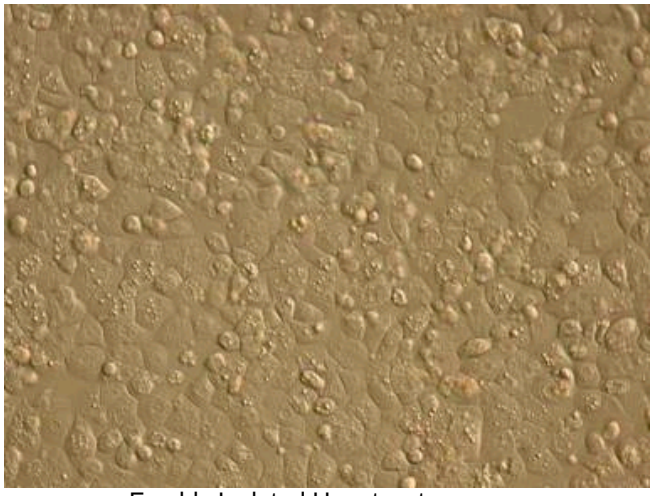
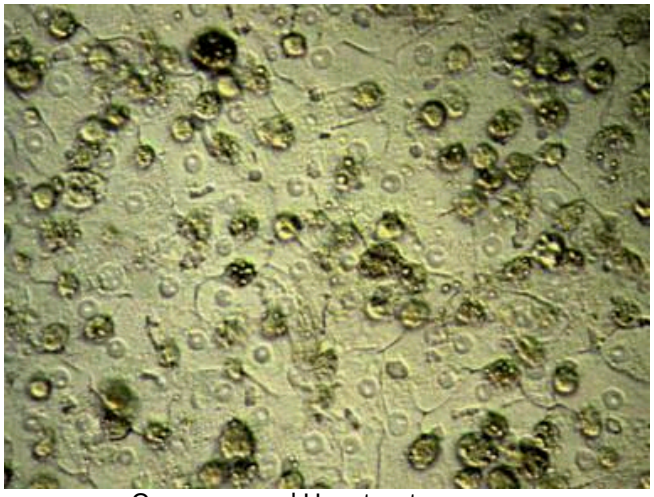
Figure 1: Representative photographs of freshly isolated and cryopreserved hepatocytes in culture.



Freshly Isolated Hepatocytes



Cryopreserved Hepatocytes

Figure 2: Concentration-response relationships for TCDD in freshly isolated human hepatocytes

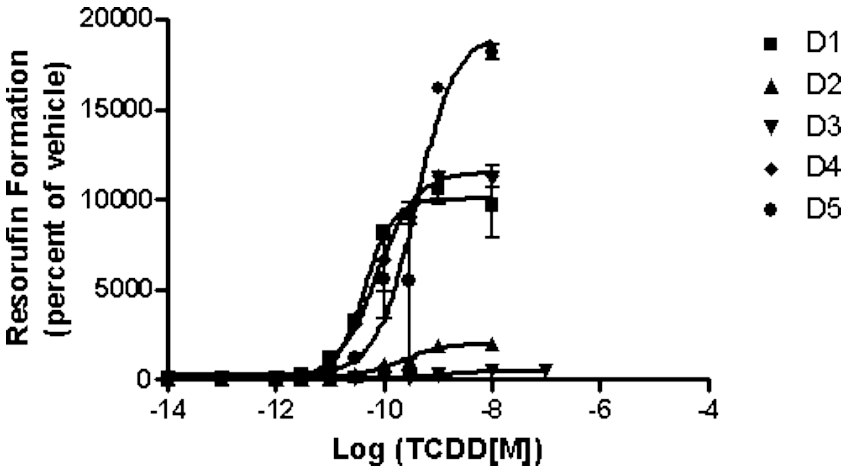


Figure 3: Concentration-response relationships for TCDD in plateable cryopreserved human hepatocytes

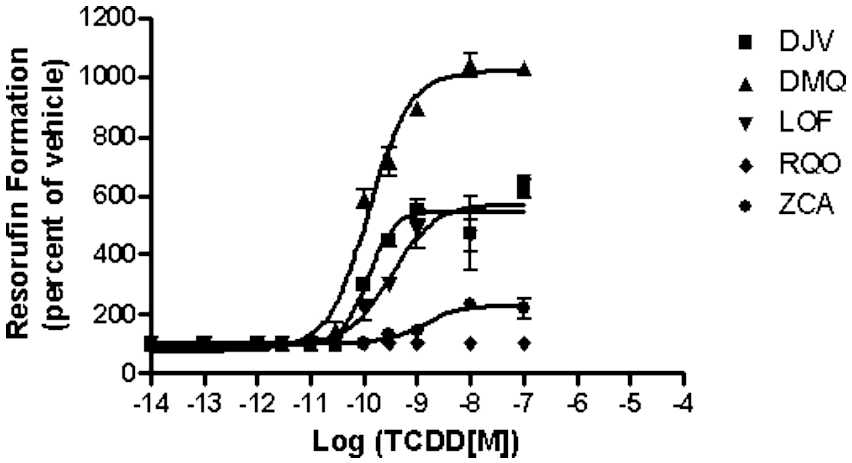


Table 1: Comparsion of EC_{50} Values for Fresh Hepatocytes from 5 Donors and Cryopreserved Hepatocytes from 5 Different Donors

EC_{50} (nM)	
Freshly isolated hepatocytes	Plateable cryopreserved hepatocytes
0.0455	0.122
0.216	0.128
1.41	0.342
0.0754	-
0.402	1.33

The mean EC_{50} value for freshly isolated hepatocytes was 0.430 ± 0.566 nM and

the mean EC_{50} value for cryopreserved hepatocytes was 0.481 ± 0.576 nM