In Vitro Cell. Dev. Biol.—Animal 34:631–635, September 1998

1071-2690/98 $05.00+0.00

ACUTE CYTOTOXICITY TESTING WITH CULTURED HUMAN LUNG AND DERMAL CELLS

FRANK A. BARILE[1] AND MONICA CARDONA

Department of Health Sciences, City University of New York at York College, Jamaica, New York 11451

(Received 15 October 1997; accepted 1 April 1998)

SUMMARY

An extensive *in vitro* study with cultured cells was conducted to test the basal cytotoxicity theory. This theory suggests that most chemical injury, at least *in vitro*, is a manifestation of one or more insults to the basic cellular structures and functions common to mammalian cells. This accounts for the similarity of results in multilaboratory studies. Human fetal lung fibroblasts (HFL1), and human skin fibroblasts (WS1, Detroit551) were studied in culture to evaluate their potential to screen for cytotoxicity. Confluent monolayers were incubated in the absence or presence of increasing concentrations of test chemicals for 24 h, and the MTT assay was used to assess toxicity. Inhibitory concentrations were extrapolated from concentration-effect curves after linear regression analysis. Twenty-nine chemicals were tested with each cell line and the cytotoxicity data compared to rodent and human lethal concentrations. The data suggest that the experimental IC_{50} values are as accurate predictors of human toxicity as equivalent toxic blood concentrations derived from rodent LD_{50}s. In addition, lung and skin fibroblasts revealed no significant differences among the three cell lines. The results support the conclusion that finite cell lines of human origin have the potential for screening chemicals for human toxicity. In combination with previously published reports, the data suggest that a basal cytotoxic phenomenon may explain the similarity of results among different human cell lines.

Key words: in vitro cytotoxicity; MTT assay; cell culture; human lung fibroblasts; human dermal fibroblasts; basal cytotoxicity.

INTRODUCTION

The past decade has seen a tremendous effort to develop *in vitro* methods in toxicology to supplement or substitute for certain animal tests for general toxicity. The development of these new *in vitro* tests must be independently reliable and sensitive, that is, able to produce consistent results as well as detect low-level toxic insult. In addition, the tests must withstand the process of validation, either independently or through interlaboratory evaluation, if they are to become part of acceptable protocols.

Recently, we have studied a variety of chemicals in several cell culture systems using different *in vitro* assays (3,4,16). In addition, the results of cytotoxicity tests of 50 chemicals was submitted to the Multicenter Evaluation for *In Vitro* Cytotoxicity program (MEIC) as part of an international multilaboratory project (9). Independently, our studies demonstrated that 50% inhibitory concentrations (IC_{50} values) determined *in vitro* with protein synthesis, MTT, and cell growth as indicators of cytotoxicity, are as accurate in screening chemicals for human toxicity as human equivalent toxic blood concentrations (HETCs) derived from established rodent LD50 values. Interlaboratory comparisons suggest that a variety of *in vitro* methods from different laboratories predicted human chemical toxicity with good conformity of data (5,10,11).

Comparisons between *in vitro* and *in vivo* data rely on the statistical methods available to convert rodent oral and parenteral LD_{50}s to acceptable blood concentrations. Thus, the calculation of HETCs is crucial for equating our *in vitro* information with rodent and human estimates of toxicity. Using volume of distribution and known rodent LD_{50} values for each chemical, a toxic plasma concentration is derived. This number is compared to experimental *in vitro* IC_{50}s as well as clinical human lethal concentrations derived from poison control information centers. It is important to note, however, that this value is an estimate since it is based on the accuracy of oral or parenteral animal dosing. In addition, it presumes that the available number for volume of distribution is uniform for each chemical for the animal species tested.

In this study, we tested 29 chemicals with lung and dermal cell lines in an attempt to show that *in vitro* methods are comparable to traditional whole animal toxicity tests in their ability to screen for cytotoxicity. We compared the data for each cell line to each other and with known human lethal plasma concentrations available for the chemicals. Also, the *in vitro* data were compared to LD_{50} values already established for these chemicals in order to examine the ability of our methods to screen for human toxicity.

This information in combination with previous results allows us to test the basal cytotoxicity theory (that is, the toxicity of a chemical to basic cellular functions and structures common to all human specialized cells) (1,13), and to evaluate the potential of the technique to screen for and predict acute human chemical toxicity.

MATERIALS AND METHODS

Cell culture. Cell culture supplies were obtained from GIBCO (Grand Island, NY). Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and from Alfa Products (Ward Hill, MA). Human fetal lung fibroblasts (HFL1, CCL-153) and human dermal fibroblasts [Detroit551 (DET), CCL-110; WS1, CRL-1502, American Type Culture Collection, Rockville, MD]

[1]To whom correspondence should be addressed.

were seeded in 96-well plates at 10^4 cells/cm^2. Cultures were grown and subcultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum as previously described (2,17). Cell lines were karyotyped upon initiation of a culture and once during the experiments to establish identity and monitor their differentiated state (data not shown). In our laboratory, finite human fibroblasts typically reach the stationary phase 6 to 8 d after seeding (6,7).

MTT assay. Confluent monolayers were incubated with each chemical in the stationary phase on Day 7 or 8 and prepared for the MTT assay (4,9,16). In a 96-well plate, cells were exposed to increasing concentrations of the chemical [8 rows, each row representing separate increasing concentrations, 12 wells (replicates) per group] for 24 h at 37° C in an atmosphere of 10% CO_2/90% air. Incubating medium consisted of modified Eagle's medium without glutamine (GIBCO's "Autoclavable MEM") supplemented with sodium bicarbonate, dialyzed fetal bovine serum (2%), and ascorbic acid (50 μg/ml). After 22 h, 10 μl MTT solution (5 mg/ml) was added to each well and incubated for an additional 2 h. The medium was replaced with 100 μl dimethyl sulphoxide (DMSO), agitated for 5 min at 25° C, and the absorbances were read at 550 nm. Cell viability was expressed as a percentage of the control groups. Additional wells with media and chemical only (without cells) were processed in parallel to test for a chemical's ability to reduce MTT directly. Although none of the chemicals demonstrated any significant reduction of MTT, the absorbance from this control group was used as the reference blank.

Dosage-range experiments were performed in all assays. Each experiment was repeated at least three times, and each dosage group was assayed in triplicate. The 50% inhibitory concentrations were extrapolated from concentration-effect curves by linear regression analysis. When the IC_{50} was not bracketed in the initial dosage range used for the chemical, the experiments were repeated and the concentrations were adjusted as necessary.

Solubilization of chemicals. Most of the experimental problems involved the solubilization or miscibility of the chemicals with the media. Solid chemicals that were insoluble in water, such as paracetamol (acetaminophen), acetylsalicylic acid (aspirin), digoxin, and 2,4-dichlorophenoxyacetic acid (2,4-DCP), presented with dissolution problems, especially at the higher dosage levels. The solubility of these chemicals was improved by micronization. A stock solution was prepared in 95% ethanol or DMSO and the solution was evaporated to dryness. The remaining lyophilized powder was then dissolved more readily in media with constant stirring at 37° C for 1 h before incubation. This method of dissolving the chemicals and evaporation of the intermediate solvents ensures that the cells are never exposed to any concentration of ethanol or DMSO.

Other organic chemicals, including xylene and malathion, were immiscible with water and the labeling medium. Miscibility was improved by sonicating a stock solution of the chemical in medium (Ultrasonic Processor W-225, Heat Systems-Ultrasonics, Farmingdale, NY) for 10 sec at 30 W. This manipulation completely homogenized the mixtures and allowed enough time for adequately dispersing an aliquot into labeling medium. Also, the more volatile chemicals were especially annoying because they permeated the incubator atmosphere and interfered with the control wells, resulting in curling of the cell layers. We controlled evaporation, and thus the concentration of the chemical in the medium, by incubating the chemicals with cells grown in 25-cm^2 (T-25) flasks instead of 96-well plates, which were then overlaid with mineral oil, individually gassed with 10% CO_2 in air, and sealed with screw caps. This pressure-tight container system was used for each volatile chemical in parallel with 96-well plate studies.

Calculation of HETC values. To facilitate comparisons with known human toxicity data, rodent LD50 values (21) were converted to human equivalent toxic concentrations according to the following formula (4,16):

$$\text{HETC} = (LD_{50})/V_d \times 10^{-3}$$

where HETC = estimated human equivalent toxic concentration in plasma (mg/ml), LD_{50} = 50% lethal dose in rodents (mg/kg, intraperitoneal or oral), V_d = volume of distribution (l/kg), and 10^{-3} = constant for conversion into ml (l/ml). The HETC values and the *in vitro* IC_{50}s for each chemical were plotted separately against human lethal concentrations to compare their ability for screening chemicals with potential for human toxicity (*see* Figures).

Statistical analysis. IC_{50} values were extrapolated from concentration-effect curves with linear regression analysis. When the IC_{50} was not bracketed by the dosage range used for the chemical, the experiment was repeated and the concentrations were adjusted accordingly. Thus, the regression was limited to the active range of the dose–response curve, following the results of the dosage-range experiments.

In addition to calculating the correlation coefficient for each concentration-effect curve (r value), we applied the hypothesis test for $\beta = 0$ (1,12,14). This computation is an alternate test of the null hypothesis of zero slope between the two variables, X (concentration) and Y (percent response) and is based on the slope of the sample regression equation. The test statistic, which is normally distributed as with the Student's t-test, is calculated and compared to minimum values of t, for $n - 2$ degrees of freedom, at the 95% and 99% confidence intervals.

Each experiment was repeated at least three times, and each dose group within an experiment was assayed in triplicate. The results from each cell line were compared to each other with single factor analysis of variance (one-way ANOVA), paired Student's t-test, and regression analysis. All statistics were performed with the Microsoft Excel® software package.

Results

In vitro inhibitory concentrations. Table 1 compares the experimental inhibitory concentrations for the first 29 chemicals determined with DET and WS1 dermal cells with data previously obtained with HFL1 cells (3). The IC50s were extrapolated from the regression plots, and their t-statistics were significant at the 95% or 99% confidence intervals. In general, the three cell lines did not show statistically significant differences in sensitivity to chemical cytotoxicity ($P > 0.05$) when compared by one-way ANOVA and the paired Student's t-test.

Fig. 1 displays the regression plots for the 24-h MTT studies comparing DET, WS1, and HFL1 cell lines. The data correspond to the values in Table 1. The regression lines for HFL1 vs. DET and HFL1 vs. WS1 have slopes of 0.90 and 0.99 and the correlation coefficients (r values) are 0.99 and 0.96, respectively. The test statistics (t = 1.92 and 1.09) show that the cell lines are not significantly different from each other ($P > 0.05$).

Fig. 2 shows the plot of the calculated human equivalent toxic blood concentrations (HETC values) derived from rodent LD_{50}s against well-defined human lethal concentrations for all 29 chemicals. The dotted line represents a theoretical slope of 1.0. The calculated value for the slope of the data (4.6, $r = 0.86$) indicates that the regression line is one order of magnitude from the theoretical slope. The plot for the experimental IC_{50}s derived with HFL1, DET, and WS1 cells vs. the same human lethal concentrations for the 30 chemicals (Figs. 3–5), show equivalent slopes and correlations (*see* figure legends).

Discussion

The results obtained from *in vitro* testing of 29 chemicals with human skin cell lines allow us to compare the information with previous human lung cell studies and with available human and animal toxicity data. From the information, we can reasonably conclude that relatively rapid *in vitro* cytotoxicity procedures have the potential for screening cytotoxic compounds as alternative methods to the traditional animal testing protocols. In addition, continuous cell lines from lung and skin do not appear to show significant differences in sensitivity to chemical toxicity.

The MTT assay, as an indicator of cell viability, presents as a sensitive and reliable indicator for detecting basal cytotoxicity, i.e., the toxicity of a chemical to basic cellular functions and structures common to all human specialized cells (1,13). Thus, mitochondrial oxidation is a process which qualitatively does not differ from other indicators of cellular metabolism, such as protein synthesis, which

TABLE 1

IN VITRO 50% INHIBITORY CONCENTRATIONS (IC_{50}S) FOR MEIC CHEMICALS 1–30: COMPARISON OF 24-HR MTT ASSAYS

Number[a]	Chemical	HFL1 cells[b]	DET cells	WS1 cells
1	Paracetamol	7.8 ± 0.8	7.1 ± 1.1	3.5 ± 0.7
2	Acetylsalicylate	3.1 ± 0.8	3.5 ± 0.7	3.1 ± 0.9
3	Ferrous sulfate	—	—	—
4	Diazepam	0.28 ± 0.05	1.02 ± 0.11	1.62 ± 0.2
5	Amitriptyline	0.028 ± 0.004	.016 ± 0.002	.021 ± 0.002
6	Digoxin	1.95 ± 0.51	3.5 ± 0.5	1.2 ± 0.2
7	Ethylene glycol	63.1 ± 7.4	61.7 ± 7.6	74.1 ± 11.0
8	Methanol	41.7 ± 4.2	33.9 ± 4.8	28.8 ± 5.2
9	Ethanol	25.1 ± 3.3	15.8 ± 2.4	13.5 ± 1.7
10	Isopropanol	5.8 ± 1.1	5.8 ± 1.2	6.2 ± 0.5
11	1,1,1-TCE Trichloroethane	4.9 ± 1.0	2.3 ± 0.7	2.3 ± 0.3
12	Phenol	1.3 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
13	Sodium chloride	7.7 ± 0.9	3.1 ± 0.6	3.3 ± 0.6
14	Sodium fluoride	0.21 ± 0.03	.46 ± 0.13	.23 ± 0.05
15	Malathion	0.62 ± 0.08	—	1.3 ± 0.4
16	2,4-dichlorophenoxyacetic acid	0.87 ± 0.22	0.89 ± 0.22	1.3 ± 0.1
17	Xylene	2.5 ± 0.4	—	0.60 ± 0.22
18	Nicotine	1.9 ± 0.4	0.96 ± 0.05	0.91 ± 0.20
19	Potassium cyanide	0.43 ± 0.06	1.1 ± 0.2	2.3 ± 0.5
20	Lithium sulfate	4.3 ± 1.1	2.8 ± 0.35	2.8 ± 0.4
21	Theophylline	0.83 ± 0.07	1.6 ± 0.3	1.5 ± 0.2
22	D-propoxyphene HCl	0.11 ± 0.02	0.115 ± 0.030	0.123 ± 0.030
23	Propranolol HCl	0.039 ± 0.006	0.027 ± 0.003	0.036 ± 0.005
24	Phenobarbital	0.38 ± 0.05	0.54 ± 0..16	0.49 ± 0.07
25	Paraquat	1.1 ± 0.1	0.63 ± 0.05	0.27 ± 0.05
26	Arsenic oxide	0.112 ± 0.013	0.019 ± 0.004	0.027 ± 0.004
27	Cupric sulfate	0.039 ± 0.006	0.014 ± 0.002	0.038 ± 0.006
28	Mercuric chloride	0.023 ± 0.003	0.017 ± 0.003	0.030 ± 0.004
29	Thioridazine HCl	0.0031 ± 0.0006	0.0046 ± 0.0006	0.0045 ± 0.0009
30	Thallium sulfate	0.15 ± 0.01	0.18 ± 0.03	0.51 ± 0.02

[a]Chemical number corresponds to MEIC list of chemicals.

[b]Data for HFL1 cells from Barile et al. (3). All values are in mg/ml ±1 SE; IC_{50} = inhibitory concentration, 50%. Comparison of the three sets of data revealed no significant differences at $P = 0.05$ with one-way ANOVA ($F = 0.04$, df = 80).

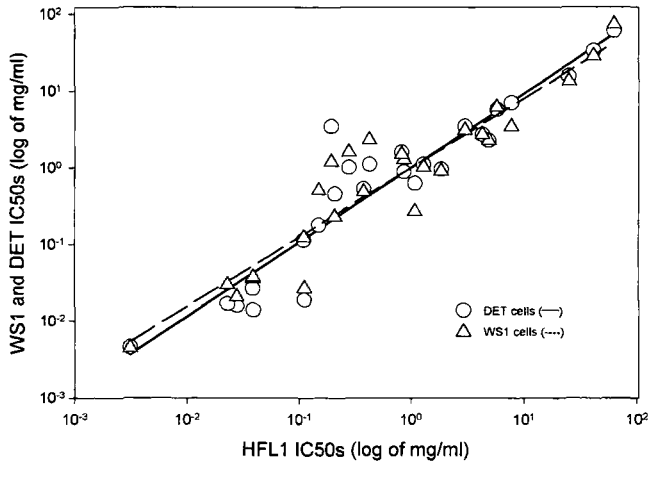

FIG. 1. Graph of 24-h MTT studies comparing HFL1, DET, and WS1 cells for chemicals 1–30. Paired comparisons of the three sets taken two sets at a time revealed no significant differences at $P = 0.05$ with a two-tailed paired Student's t-test and regression analysis (HFL1 vs. DET: $t = 1.92$, df = 26, $r = 0.99$; HFL1 vs. WS1: $t = 1.09$, df = 26, $r = 0.96$; DET vs. WS1: $t = 0.07$, df = 26, $r = 0.99$).

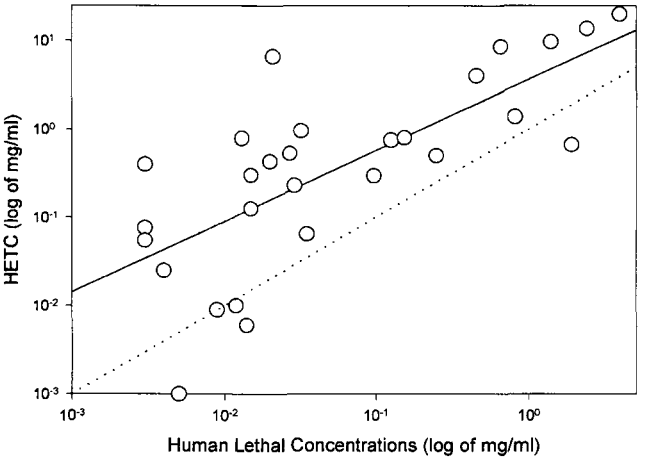

FIG. 2. Graph of human equivalent toxic concentration (HETC) values derived from rodent LD_{50}s vs. known human lethal concentrations for the 30 MEIC chemicals. The regression line (*solid*) has a slope of 4.6 ($r = 0.86$) and is significant at $P < 0.001$ ($F = 78.4$, df = 28, one-way ANOVA). The *dotted line* represents a theoretical slope of 1.0. Human lethal concentrations and HETC values were obtained from Lewis (20), Baselt and Cravey (8), and tabulated in Barile et al. (3,5).

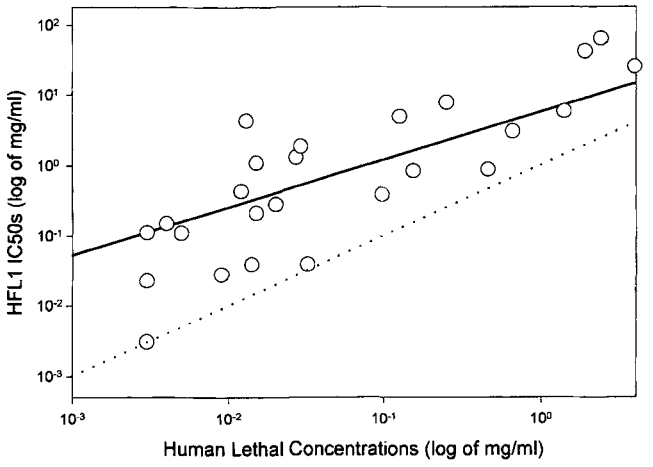

FIG. 3. Graph of experimental IC_{50}s for HFL1 cells with the MTT assay vs. known human lethal concentrations for 29 MEIC chemicals. The regression line (*solid*) has a slope of 12.1 ($r = 0.77$) and is significant at $P < 0.001$ ($F = 35.4$, df = 25, one-way ANOVA). The *dotted line* represents a theoretical slope of 1.0. Human lethal concentrations were obtained from Lewis (20), Baselt and Cravey (8), and tabulated in Barile et al. (3,13).

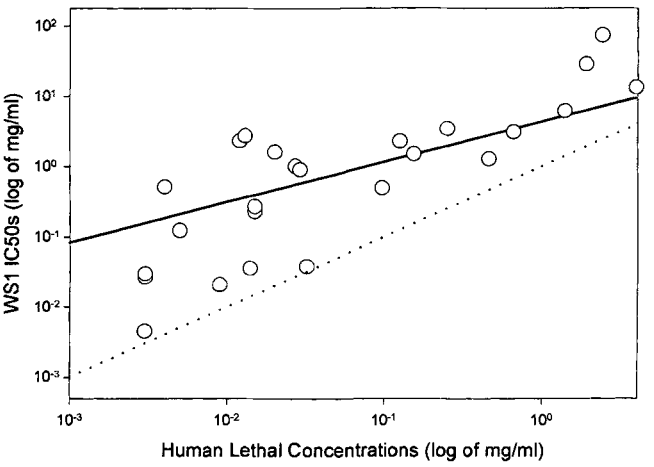

FIG. 5. Graph of experimental IC_{50}s for WS1 cells with the MTT assay vs. known human lethal concentrations for 29 MEIC chemicals. The regression line (*solid*) has a slope of 10.5 ($r = 0.65$) and is significant at $P < 0.001$ ($F = 17.8$, df = 25, one-way ANOVA). The *dotted line* represents a theoretical slope of 1.0. Human lethal concentrations were obtained from Lewis (20), Baselt and Cravey (8), and tabulated in Barile et al. (3,13).

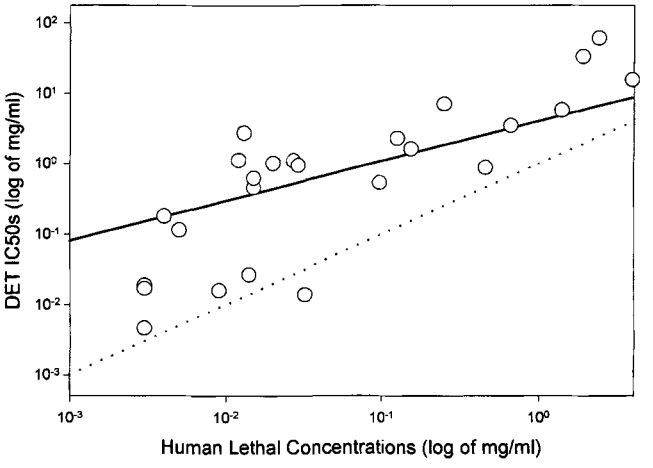

FIG. 4. Graph of experimental IC_{50}s for DET cells with the MTT assay vs. known human lethal concentrations for 27 MEIC chemicals. The regression line (*solid*) has a slope of 10.1 ($r = 0.70$) and is significant at $P < 0.001$ ($F = 23.6$, df = 25, one-way ANOVA). The *dotted line* represents a theoretical slope of 1.0. Human lethal concentrations were obtained from Lewis (20), Baselt and Cravey (8), and tabulated in Barile et al. (3,13).

we previously showed to be an analogous endpoint of cell toxicity (4,16).

One must be aware, however, that as with any testing procedure, some chemicals such as digoxin may elude the cytotoxic screen. Jover et al. (19) demonstrated that digoxin was the most cytotoxic of the first 10 MEIC chemicals to human hepatocytes. In addition, the authors demonstrated that acute toxicity in humans was more accurately predicted with human hepatocytes than with rodent hepatocytes or 3T3 cells. Wallace et al. (26) showed that digoxin was the most toxic compound of the first 10 MEIC chemicals in human epidermal keratinocytes when using the neutral red uptake assay. Because of the limits of solubility for digoxin in the culture medium, however, the investigators could not determine an IC_{50} using the neutral red release assay. In our study, digoxin showed intermediate toxicity with two human dermal fibroblast cell lines, which correlated with previous data obtained with human fetal lung fibroblasts (3,16). The difficulty encountered with solubility and the lack of significant metabolic enzyme activity in fibroblasts when compared to primary cultured hepatocytes may account for the underestimation of digoxin's toxicity. In fact, Clemedson et al. (11) described the deviating behavior of digoxin and malathion among 30 MEIC chemicals from the results of 68 *in vitro* assays. The authors concluded that human cell toxicity was better predicted with mammalian cells when digoxin was excluded from the comparisons (11).

Several recent studies by investigators from individual laboratories using MEIC chemicals have compared their results with human and animal toxicity data, using a variety of *in vitro* methods. Ponsoda et al. (23) tested 20 MEIC chemicals against the 24-h MTT assay in four rat and human cell lines. They concluded that all of the compounds showed similar acute basal cytotoxicity regardless of whether the cells were metabolically competent or of human origin. In addition, because all four systems gave similar predictions of human toxicity, they suggest that cellular systems are better predictive tools for human toxicity than are prokaryotic cells or whole organism models. Järkelid et al. (18) screened 20 MEIC chemicals in L-929 mouse fibroblast cells. A 72-h exposure using the neutral red uptake assay revealed results in accordance with previous studies conducted in our lab (17). Using three endpoints to test 10 MEIC chemicals on rainbow trout (R1) fibroblasts, Segner and Schüürmann (24) report 80% correlation in toxicity ranking between the various bioassays. They suggest that the high degree of conformance may be explained by the basal cytotoxicity concept.

In a recent review, Garle et al. (15) summarized the important cytotoxicity tests published over the previous 5 yr. They concluded that *in vitro* data correlated better with rodent LD_{50} values for certain groups of related chemicals than for some groups of unrelated sub-

stances. Seemingly unrelated endpoints, cell methods, and exposure periods appear to have a minimal effect on *in vivo* and *in vitro* correlation. They recommend that a small battery of different *in vitro* systems be used for discriminating between basal and differential cytotoxicity. More recently, participants at the ECVAM workshop (25) concluded that an *in vitro* tier testing scheme could be used for acute toxicity testing. The scheme would incorporate testing for basal cytotoxicity at stage 1, the role of biotransformation at the second stage, and methods for detecting selective cell-specific cytotoxicity at the third stage.

In a multilaboratory MEIC study (10,11), investigators at 31 laboratories using 68 different *in vitro* cytotoxicity testing methods reported that IC_{50} values from all cytotoxicity assays, in combination with toxicokinetic data (intestinal absorption and volume of distribution), predicted human lethal dosage as efficient as mouse and rat LD_{50} values for the first 30 chemicals. The MEIC study also suggested that primary cultures and human cell lines may be more sensitive to chemical toxicity than animal cell lines. In general, it appears that basal cytotoxicity can be detected in finite and continuous cell lines by a variety of techniques.

The results from our study support our previous hypothesis that most chemical injury is precipitated through a basal cytotoxic mechanism (1,13), i.e., the toxic effect is a manifestation of one or more insults to the basic cellular structures and functions common to all mammalian specialized cells. The concept of a basal cytotoxic response accounts for the similarity of results observed between three cell lines of human origin, albeit from different organs, thus representing the fundamental reaction of cells to a toxic stimulus. Consequently, the response of cultured cells to equivalent doses of a substance may reflect their ability to adapt to *in vitro* conditions, rather than their genetically programmed response from the primary site of origin. In addition, given the variability of the *in vivo* systems (both human lethal concentrations and LD_{50} results), the *in vitro* values are generally as good in predicting human cell toxicity as LD_{50} values. It is conceivable, therefore, that tests using finite differentiated cell lines of various origins could be developed to cover a large percentage of toxic effects and would reduce the need to introduce many laborious systems with specific cells.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health, NIGMS (MBRS No. GM08153).

References

1. Barile, F. A. Introduction to *in vitro* cytotoxicology: mechanisms and methods. Boca Raton, FL: CRC Press; 1994:27–32, 180–186.
2. Barile, F. A. Continuous cell lines as a model for drug toxicity assessment. In: Castell, J. V.; Gómez-Lechón, M.-J., ed. *In vitro* methods in pharmaceutical research. London: Academic Press; 1997:33–54.
3. Barile, F. A.; Alexander, D.; Sookhoo, A. Potential of human lung cells for predicting acute cytotoxicity. ATLA 23:461–468; 1995.
4. Barile, F. A.; Arjun, S.; Hopkinson, D. *In vitro* cytotoxicity testing: biological and statistical significance. Toxicol. In Vitro 7:111–116; 1993.
5. Barile, F. A.; Dierickx, P. J.; Kristen, U. *In vitro* cytotoxicity testing for prediction of acute human toxicity. Cell Biol. Toxicol. 10:155–162; 1994.
6. Barile, F. A.; Guzowski, D. E.; Ripley, C., et al. Ammonium chloride inhibits basal degradation of newly synthesized collagen in human fetal lung fibroblasts. Arch. Biochem. Biophys. 276:125–131; 1990.
7. Barile, F. A.; Siddiqi, Z.; Rouzier, C. R., et al. Effects of puromycin and hydroxynorvaline on production and intracellular degradation of collagen in human fetal lung fibroblasts. Arch. Biochem. Biophys. 270:294–301; 1989.
8. Baselt, R. C.; Cravey, R. H. Disposition of toxic drugs and chemicals in man. 3rd edition. Chicago: Year Book Medical Pub., Inc.; 1989.
9. Bondesson, I.; Ekwall, B.; Hellberg, S., et al. MEIC—a new international multicenter project to evaluate the relevance to human toxicity of *in vitro* cytotoxicity tests. Cell Biol. Toxicol. 5:331–348; 1989.
10. Clemedson, C.; Mcfarlane-abdulla, E.; Andersson, M., et al. MEIC evaluation of acute systemic toxicity. Part I. Methodology of 68 *in vitro* toxicity assays for the first 30 reference chemicals. ATLA 24:251–272; 1996.
11. Clemedson, C.; Mcfarlane-abdulla, E.; Andersson, M., et al. MEIC evaluation of acute systemic toxicity. Part II. *In vitro* results from 68 toxicity assays used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. ATLA 24:273–311; 1996.
12. Daniel, W. W. Biostatistics: a foundation for analysis in the health sciences. 2nd edition. New York: John Wiley and Sons; 1978.
13. Ekwall, B. Basal cytotoxicity data (BC-data) in human risk assessment. In: Proceedings of the Workshop on Risk Assessment and Risk Management of Toxic Chemicals. Ibaraki, Japan: National Institute for Environmental Studies; 1992:137–142.
14. Gad, S.; Weil, C. S. Statistics and experimental design for toxicologists. 2nd ed. Caldwell, NJ: Telford Press; 1988:55–59, 102–104.
15. Garle, M. J.; Fentem, J. H.; Fry, J. R. In vitro cytotoxicity tests for the prediction of acute toxicity *in vivo*. Toxicol. In Vitro 8:1303–1312; 1994.
16. Hopkinson, D.; Bourne, R.; Barile, F. A. *In vitro* cytotoxicity testing: 24-hour and 72-hour studies with cultured lung cells. ATLA 21:167–172; 1993.
17. Hopkinson, D.; Scheiner, P.; Barile, F. A. *In vitro* cytotoxicity testing of potentially active anti-HIV drugs with cultured cells. ATLA 24:413–418; 1996.
18. Järkelid, L.; Kjellstrand, P.; Martinson, E., et al. Toxicity of 20 chemicals from the MEIC programme determined by growth inhibition of L-929 fibroblast-like cells. ATLA 25:55–59; 1997.
19. Jover, R.; Ponsoda, X.; Castell, J. V., et al. Evaluation of the cytotoxicity of ten MEIC chemicals on human cultured hepatocytes: predictability of human toxicity and comparison with rodent cell culture systems. Toxicol. In Vitro 6:47–52; 1992.
20. Lewis, R. J. Hazardous chemicals desk reference. 3rd edition. New York: Van Nostrand Reinhold; 1993.
21. Merck Index. Budavari, S.; O'Neil, M. J.; Smith, A., et al., ed. 11th ed. Rahway, NJ: Merck and Co. Inc.; 1989.
22. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65:55–63; 1983.
23. Ponsoda, X.; Nunez, C.; Castell, J. V., et al. Evaluation of the cytotoxic effects of MEIC chemicals 31–50 on primary culture of rat hepatocytes and hepatic and non-hepatic cell lines. ATLA 25:423–436; 1997.
24. Segner, H.; Schüürmann, G. Cytotoxicity of MEIC chemicals to rainbow trout R1 cell line and multivariate comparison with ecotoxicity tests. ATLA 25:331–338; 1997.
25. Seibert, H.; Balls, M.; Fentem, J. H., et al. Acute toxicity testing *in vitro* and the classification and labelling of chemicals. ATLA 24:499–510; 1996.
26. Wallace, K. A.; Harbell, J. W.; Accomando, N., et al. Evaluation of the human epidermal keratinocyte neutral red release and neutral red uptake assay using the first 10 MEIC test materials. Toxicol. In Vitro 6:367–371; 1992.