Cancer Chemother Pharmacol (1999) 43: 59–67

ORIGINAL ARTICLE

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Use of V79 cells with stably transfected cytochrome P450 cDNAs in studying the metabolism and effects of cytotoxic drugs

Received: 18 December 1997 / Accepted: 14 May 1998

Abstract *Purpose*: Studying the metabolism of cytotoxic drugs has become increasingly necessary to predict clinically significant drug-drug interactions and to understand the basis of interindividual variations in the pharmacokinetics of anticancer agents. The aim of this study was to determine the feasibility of using V79 Chinese hamster fibroblasts, which are stably transfected with cytochrome P450 (CYP) cDNAs, to study the metabolism of cytotoxic drugs in vitro. *Methods*: The 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell survival after incubation with drugs. Gas chromatography/mass spectroscopy was used for the quantitation of metabolites of cyclophosphamide and ifosfamide in culture medium. The coculture technique was used to study the generation of cytotoxic metabolites in culture medium. *Results*: After treatment with either cyclophosphamide or ifosfamide (100 μ*M* to 1 m*M*) cytotoxicity was demonstrated in only cytochrome CYP2B1- and cytochrome CYP3A4-expressing cells. Treatment of parental nontransfected cells that were cocultured with CYP-expressing cells with cyclophosphamide resulted in increased sensitivity to this drug. All active and inactive metabolites of cyclophosphamide and ifosfamide were detected in the culture medium. Cyclophosphamide-induced cytotoxicity in CYP2B1- and CYP3A4-expressing cells was abrogated by metyrapone and midazolam/troleandomycin, respectively. Paclitaxel showed greater cytotoxicity against parental V79 cells than against the CYP2B1-, 2E1-, or 3A4-expressing cells, which was also influenced by cotreatment with CYP inhibitors. *Conclusions*: Stable expression of *CYP* cDNAs by V79 cells provided an in vitro system to study cytotoxic drug metabolism. Cell viability and metabolite assays were used to determine the differential metabolism and effects in different CYP-transfected cell lines treated with cytotoxic drugs. The potential use of this V79 cell expression system is in studying enzymes involved in the metabolism of cytotoxic drugs, especially early in drug development. In addition, this system may be used to determine drug interactions that may influence the outcome of therapy in patients with cancer.

Key words Cytochrome P450 · Cyclophosphamide · Drug metabolism · Ifosfamide · Paclitaxel · V79 cells

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Introduction

The microsomal cytochrome P450 (CYP) drug-metabolizing enzymes are a superfamily of hemoproteins responsible for the oxidative metabolism of a wide variety of substrates of diverse chemical structure, including chemical carcinogens and cytotoxic drugs [17]. It is important to determine at an early stage of drug development the relative contributions of metabolism and excretion in the elimination of a drug. If metabolism were the primary or a major route of elimination it would then be necessary to identify the major enzyme(s) involved in the metabolic pathways. This knowledge would help elucidate the interindividual variations in pharmacokinetics of cytotoxic drugs but more importantly help predict clinically significant drug interactions.

Human hepatic microsomes, mostly from biopsies of diseased livers, have provided the most convenient way

to study CYP metabolism in vitro. Cultured hepatocytes from similar human sources have become an increasingly popular tool to investigate drug metabolism in vitro [14]. More recently, and with the cloning of various *CYP* genes, cellular expression systems have been developed to study the metabolism of various chemicals in vitro [7, 8]. Such CYP-expressing cells have been primarily developed for toxicological applications to study the mutagenicity of chemical procarcinogens that require metabolic activation for biologic activity [12]. These systems may overcome problems arising in the proper allocation of metabolic pathways to specific isoforms by conventional techniques (e.g. hepatocytes) which include a mixture of CYPs. The V79 Chinese hamster lung fibroblast is one such expression system that has been most widely used in mutagenicity studies. The parental cells have no CYP-dependent enzymatic activity but contain NADPH-dependent cytochrome P450 reductase, which is the enzyme required for CYPs to be operative as monooxygenases. Previous studies have established the potential use of V79 cells in studying the metabolism of cytotoxic drugs [10, 11].

We sought to further characterize the drug-metabolizing activities of these cells and to investigate their utility in studying the metabolism of anticancer drugs using cell survival and metabolite assay as the primary end-points for these experiments. To achieve these aims we tested the cytotoxicity of cyclophosphamide and ifosfamide that require metabolic activation, and paclitaxel that is metabolically inactivated by CYPs.

Materials and methods

Drugs and chemicals

Cyclophosphamide, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), metyrapone (MTP), and troleandomycin (TAO) were purchased from Sigma Chemicals (St. Louis, Mo.). Bristol-Myers Squibb (Princeton, N.J.) kindly donated ifosfamide (Ifex) and paclitaxel (Taxol). Midazolam (Versed) was a gift from Roche Laboratories (Nutley, N.J.). All deuterium-labeled internal standards, specifically CP-d_8 and phosphoramide mustard-d8 (PM-d_8) [2], ifosfamide-d_6, 4-hydroxyifosfamide-d_6, iphosphoramide mustard-d_6 (IPM-d_6), N2-dechloroethylifosfamide-d4 (N2D-d_4), N3-dechloroethylifosfamide-d_4 (N3D-d_4) [25], and N-2-dechloroethyl aziridine-d_4 (CEA-d_4) [16] were supplied by the Ohio State University Laboratory and prepared by published methods. Sodium phosphate, citric acid, isopropanol, and methanol were purchased from Sigma Chemicals.

Cell lines and culture

Chinese hamster lung fibroblast V79 cells stably expressing CYP enzymes were used in this study. The parental cells were transfected with cloned full-length *CYP* cDNA. Murine *CYP2B1* [9], human *CYP2E1* [20], and human *CYP3A4* [21] cDNAs were used in the development of these cell lines. Stable high level expression of CYP protein was achieved using a recombinant plasmid vector containing the simian virus (SV40) early promoter reading into the DNA encoding the *CYP* cDNA. Maximum CYP expression was obtained at a cell confluency of 60–80% with cell feeding every second day.

V79 cells were grown as a monolayer cell culture at 37 °C in DMEM (GIBCO BRL, Gaithersburg, Md.) supplemented with L-glutamine (1 m*M*), 10% (vol/vol) fetal bovine serum (Sigma Chemicals) and G418 (400 μg/ml). The nontransfected parental cells were grown in the presence of 1% penicillin/streptomycin instead of G418. Cultures were grown at 37 °C in a humidified atmosphere of 5% CO_2/95% air. V79 cells double every 10–12 h and were subcultured at a 1:20 ratio approximately every other day. Cells at third to fifth passage were routinely used for experiments. Cells were kept from reaching confluence at any time for optimal cell physiological conditions and maximum CYP activity. All culture media and growth factors were purchased from (GIBCO-BRL, Grand Island, N.Y.) unless stated otherwise.

Drug treatment

Cells in exponential growth phase were washed with Hank's balanced salt solution. Depending on the cell line and experiment, 10 000 to 50 000 cells/well were seeded in duplicate in 16-mm wells (Costar, Ann Arbor, Mich.). Drugs were added at increasing concentrations 24 h after plating. On the third day, the drug-containing medium was replaced by fresh medium and incubation was continued for another 24 h. Control cells were treated with an equal concentration of vehicle alone, usually not exceeding 0.1% (vol/vol). Enzyme inhibition studies were undertaken using known substrates for CYP2B1 (MTP) and CYP3A4 (TAO and midazolam). TAO was dissolved in DMSO whereas MTP and midazolam were in methanol solution.

MTT assay

Cell viability was determined by the standard MTT reduction assay with slight modifications [1]. This is a colorimetric assay based on the ability of live but not dead cells to reduce a tetrazolium-based compound (MTT) to a blue formazan product. Aliquots of 250 μl MTT (1 mg/ml) were added to each well. After incubation for 1 h at 37 °C, the supernatant was removed and 500 μl isopropanol was then added. The plates were vigorously shaken for 30 min to solubilize the MTT-formazan product. Aliquots of 200 μl were transferred into 96-well culture plates. The readings were recorded on an MR 700 microplate reader (Dynamic Laboratories, Chantilly, Va.) at 540 nm. Vehicle-treated cells were assigned a value of 100%.

Quantitation of cyclophosphamide and ifosfamide and metabolites

The generation of metabolites into culture medium from cells treated with either cyclophosphamide or ifosfamide was investigated using a range of substrate concentrations (100–5000 μ*M*) and incubation times (4–20 h). The number of cells per well was 100 000. The deuterated analogs of each analyte were used as its internal standard, except for 4-hydroxycyclophosphamide for which 4-hydroxyifosfamide-d_6, was used because of lack of supply of the labeled analog of the former. No difference was found using either labeled analog as the analyte. KCN at 0.1 *M* was used as the stabilizing agent for the ring hydroxylated metabolites with adjustment of the pH to 8.0 using $NaHSO_4$. A 1-ml aliquot of culture medium was added to respective vials and stored frozen pending analysis.

Quantitation of cyclophosphamide and metabolites was undertaken using gas chromatography and mass spectroscopy [2]. The previously published method was modified to include the assay of the metabolite N-2-chlorethylaziridine (CEA) [19]. This metabolite of cyclophosphamide is thought to be formed directly via the phosphoramide mustard, which spontaneously forms the aziridium ion [6] that readily undergoes cleavage to CEA [16]. Briefly, a 0.4-ml aliquot of the culture medium was placed in a tube containing the internal standards and the content extracted with 5.0 ml methylene chloride. After evaporation, the residue was analyzed for

cyclophosphamide and 4-hydroxycyclophosphamide. To another 0.2 ml from the same sample CEA-d_4 was added and the content extracted using 3.0 ml methylene chloride. Quantitation of ifosfamide and metabolites was accomplished using a previously reported method [25].

Coculture experiments

The bystander cytotoxic activity of metabolically active cells treated with cyclophosphamide was studied in a coculture system. CYP2B1- or CYP3A4-expressing cells were cocultured with non-transfected V79 cells. V79 CYP2B1- or CYP3A4-expressing cells (50 000) were plated in duplicate in 32-mm six-well plates. V79 cells were plated onto 0.4 μm × 30 mm culture plate inserts (Sigma Chemicals). After an overnight incubation, inserts with V79 were transferred into the culture plates containing V79 CYP2B1 or CYP3A4 cells. Cocultured cells were then treated with cyclophosphamide (100 μ*M* to 5 m*M*) for 20 h. MTT was subsequently determined as described above.

Results

Cytotoxic effects of treatment with cyclophosphamide and ifosfamide

Treatment of the parental V79 cells and cells transfected with *CYP* cDNAs with either cyclophosphamide or ifosfamide (100 μM to 1 m*M*) resulted in cytotoxicity in cells expressing CYP2B1 and CYP3A4 enzymes (Fig. 1A,B). The parental V79 cells and those expressing CYP2E1 were relatively resistant to the both drugs. For a given drug concentration, cytotoxicity was higher in cells transfected with *CYP2B1* cDNA compared to those expressing CYP3A4. CYP inhibitors were used to confirm the enzyme-specific cytotoxicity demonstrated in the CYP2B1- and CYP3A4-expressing cells. MTP, a nonspecific inhibitor of CYP2B1, partially reversed cytotoxicity in CYP2B1-expressing cells whereas midazolam and TAO (CYP3A4 substrates) fully reversed cytotoxicity in cells expressing CYP3A4 but not in those expressing CYP2B1 (Fig. 2). When used alone, these inhibitors were not cytotoxic to any of these cell lines (data not shown). Concentration-dependent cytotoxicity by DMSO alone was noted in the V79 cells (data not shown). All experiments were therefore conducted with final DMSO concentrations not exceeding 0.1% (v/v).

Quantitation of cyclophosphamide and ifosfamide metabolites in culture medium

The generation of metabolites by cyclophosphamide-treated cells is shown in Fig. 3. There was some metabolic activity in the parental cells with the generation of 1 μ*M* of 4-hydroxy metabolite when incubated with cyclophosphamide 5000 μ*M* (data not shown). No appreciable CEA levels were detected, however. In cells transfected with CYP2B1, significant metabolic activity of cyclophosphamide (4-hydroxy and phosphoramide mustard) was found and the activity increased with time and with an increase in substrate concentration. In general, maximal levels of the metabolites were reached at 8 h exposure time. The phosphoramide mustard levels almost quantitatively reflected cyclophosphamide activation. Interestingly, the cytotoxic cyclophosphamide metabolite CEA was detected at substantial levels and the generation of this metabolite from cells was demonstrated for the first time. In V79 cells expressing CYP3A4, there was also significant increase in 4-hydroxylation albeit at about two-thirds lower levels when compared with the CYP2B1-transfected cells. Additionally, phosphoramide mustard and CEA levels were also lower than those from CYP2B1-expressing cells.

The metabolism of ifosfamide by the V79 cells lines is shown in Fig. 4. There was some activation in the parental cells (data not shown). In the CYP2B-expressing cells, activation of ifosfamide occurred in a time- and

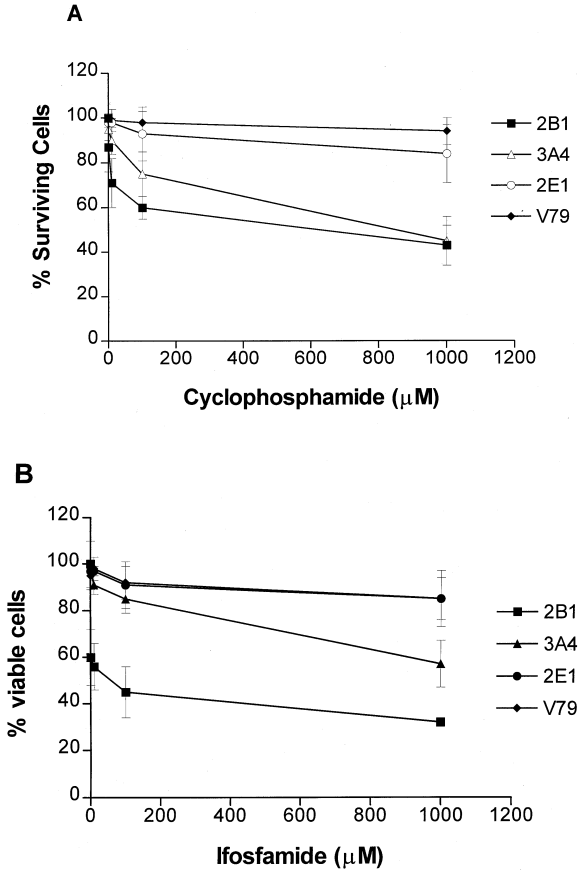


Fig. 1A,B Survival curves of cells treated with either cyclophosphamide (**A**) or ifosfamide (**B**). Parental V79 cells and those transfected with one of three CYPs were incubated with increasing concentrations of either drug for 20 h. Cytotoxicity was determined by the MTT assay and the percentage surviving cells relative to respective PBS-treated controls was calculated for each concentration of drug. Significant cytotoxicity was demonstrated in cells expressing CYP2B1 and CYP3A4. Minimal cell kill was seen in parental cells and cells expressing CYP2E1. These results indicate that CYP2B1 and CYP3A4 are involved in the activation of both cyclophosphamide and ifosfamide (*bars* ± SD)

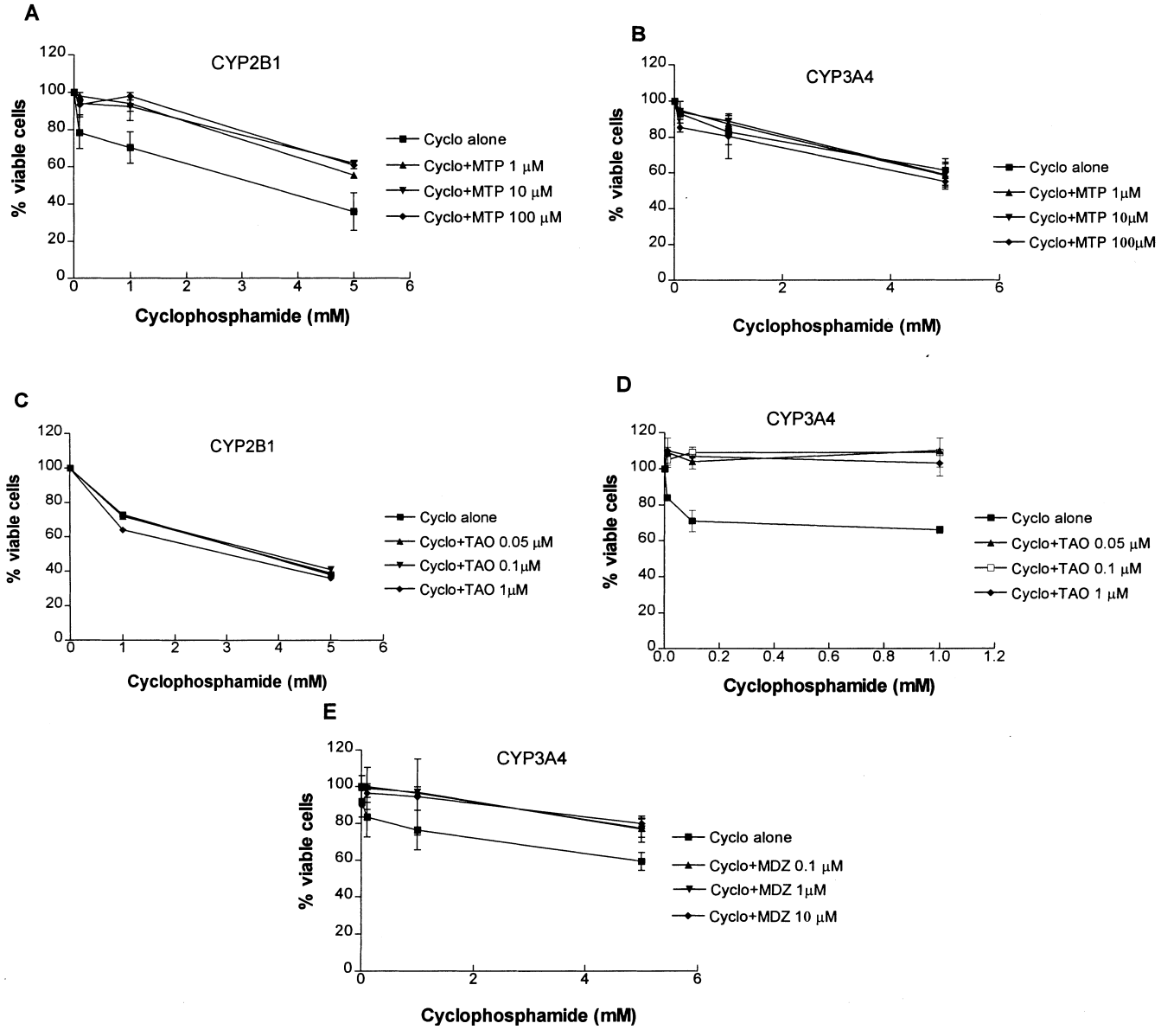


Fig. 2A–E The influence of coincubation with CYP substrates on the cytotoxicity induced by cyclophosphamide in CYP-expressing cells. Cell survival was based on the MTT assay and the percentage survival relative to PBS-treated cells was determined. **A**, **B** CYP2B1-expressing cells; **C**, **D**, **E** CYP3A4-expressing cells (*Cyclo* cyclophosphamide, *MDZ* midazolam, *MTP* metyrapone, *TAO* treolandomycin). The values are the means of three experiments (*bars* ±SD)

substrate concentration-dependent manner, so that maximal metabolic activity was reached at 8 h and at a concentration of substrate of 5000 μ*M*. Iphosphoramide mustard levels essentially reflected those of 4-hydroxy-ifosfamide. There was also a high detoxification activity in these cells with the generation of N2D and N3D metabolites in CYP2B1- and CYP3A4-expressing cells. In the CYP3A4-transfected cells, activation of ifosfamide was rapid and higher than ifosfamide metabolism by the CYP2B1 cells but less than that for cyclophosphamide. Detoxification of ifosfamide was somewhat similar in CYP2B1- and CYP3A4-expressing cells. However, at later time-points, N3D was generated at higher levels than N2D.

Cytotoxicity in V79 cells cocultured with CYP2B1- or CYP3A4-expressing cells treated with cyclophosphamide

The cytotoxicity towards V79 cells in a coculture system using CYP2B1- or CYP3A4-expressing cells was investigated. Whereas treatment of parental cells with cyclophosphamide resulted in minimal cell death (Fig. 1A), the presence of cocultured CYP2B1- or CYP3A4-expressing cells, but not parental cells, increased the sensitivity of the parental cells to cyclophosphamide (Fig. 5A–C). These results indicate that the cytotoxicity

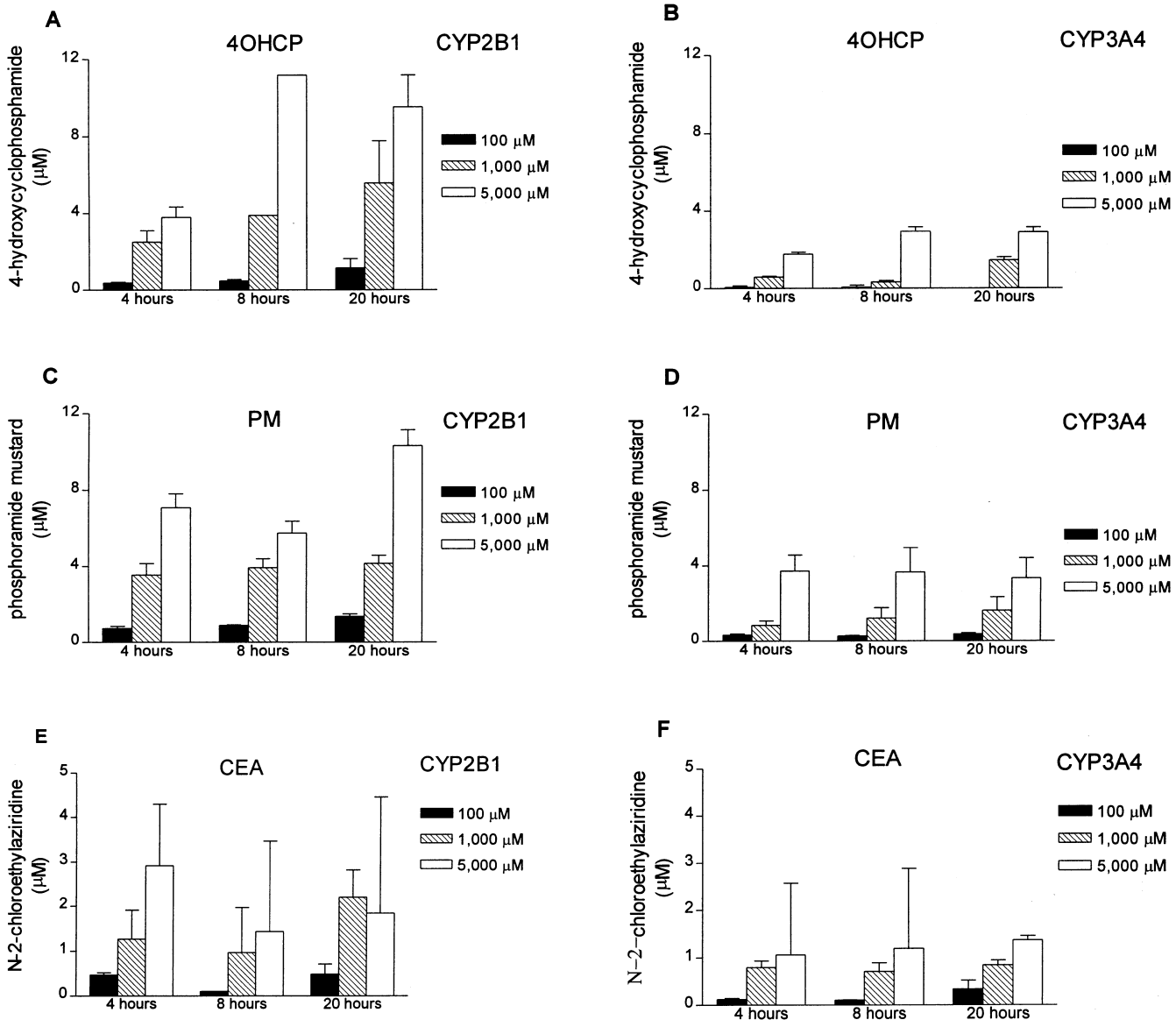


Fig. 3A–F Generation of metabolites from incubation of cyclophosphamide with V79 cells expressing either murine CYP2B1 (**A**, **C**, **E**) or human CYP3A4 (**B**, **D**, **F**) enzymes. Cells were plated at a density of 100 000 per well. Cyclophosphamide concentrations in the culture medium were 100 μ*M* to 5 m*M* and three incubation times (4, 8, and 20 h) were used. The concentrations of metabolites in the culture medium at the end of incubation periods were determined by GC/MS as described in Materials and methods. Values are the means of two experiments +SD

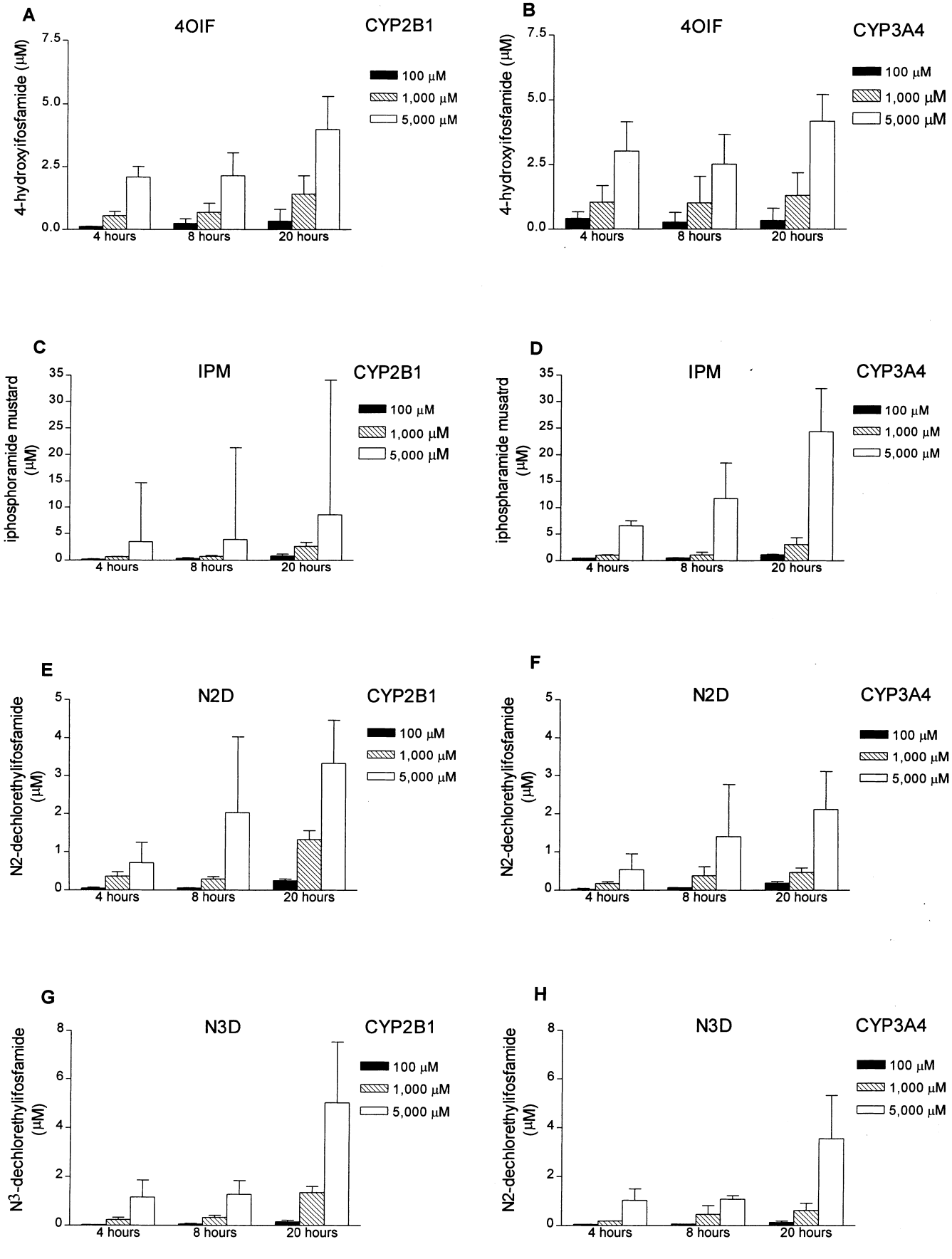
towards the parental V79 cells was due to a bystander effect of metabolically active cells treated with cyclophosphamide.

Treatment of cells with paclitaxel

Unlike cyclophosphamide or ifosfamide, paclitaxel is not known to require metabolic activation for cytotoxicity and was cytotoxic to the parental V79 cells (Fig. 6). However, cytotoxicity was less in cells expressing the CYP2B1, CYP2E1, or CYP3A4 enzymes. Cotreatment of cells with paclitaxel and either MTP (CYP2B1 inhibitor) or TAO (CYP3A4 inhibitor) resulted in a reduction in cytotoxicity towards CYP2B1- and CYP3A4-expressing cells, respectively (Fig. 7). Taken together, these results indicate that the metabolism of paclitaxel was probably responsible for the differential cell kill in these cell lines.

Discussion

The results of this study show that the expression of CYPs 2B1 and 3A4 but not 2E1 in V79 cells treated with cyclophosphamide or ifosfamide resulted in concentration-dependent cytotoxicity. These results are in agreement with those of previous studies using rat [24] and human liver microsomes and cell expression sys-


A
4OIF
CYP2B1
4-hydroxyifosfamide (μM)
7.5
5.0
2.5
0.0
4 hours
8 hours
20 hours
100 μM
1,000 μM
5,000 μM
B
4OIF
CYP3A4
4-hydroxyifosfamide (μM)
C
IPM
CYP2B1
iphosphoramide mustard (μM)
35
30
25
20
15
10
5
0
D
IPM
CYP3A4
iphospharamide musatrd (μM)
E
N2D
CYP2B1
N2-dechlorethylifosfamide (μM)
F
N2D
CYP3A4
G
N3D
CYP2B1
N3-dechlorethylifosfamide (μM)
H
N3D
CYP3A4
N2-dechlorethylifosfamide (μM)

◀

Fig. 4A–H Concentrations of ifosfamide metabolites in culture medium from CYP2B1- (**A**, **C**, **E**, **G**) or CYP3A4- (**B**, **D**, **F**, **H**) expressing cells (100 000 cells per well). Final concentrations of ifosfamide in the culture medium were 100 μ*M* to 5 m*M*. Cells were treated for 4, 8 or 20 h. The concentrations of metabolites in the culture medium at the end of each treatment period was determined by GC/MS as described in Materials and methods. The values are the means of two experiments with + SD

tems [5] and confirm the role of CYPs 2B1 and 3A4 in cyclophosphamide and ifosfamide metabolism. For a given drug concentration, cytotoxicity was higher in 2B1- than 3A4-expressing cells (Fig. 1) which is also in agreement with previously reported results [3]. However, this may also result from the higher expression of CYP protein in CYP2B1 cells compared to those transfected with CYP3A4. It is noteworthy that with a sensitive GC/MS analytical method it was possible to detect all of the known active and inactive metabolites of cyclophosphamide and ifosfamide in culture medium (Fig. 3 and 4). The design of this study did not permit conclusions regarding the differential enzymatic efficiencies of the two CYPs for the metabolism of either drug. Previous studies using human liver microsomes have also shown that CYP2B and CYP3A preferentially catalyze the 4-hydroxylation of cyclophosphamide and ifosfamide, respectively [3, 5, 24]. This study also confirms the involvement of CYP2B1 and CYP3A4 in the generation of the N2D and N3D metabolites of ifosfamide. These findings are in agreement with those of other recent studies using different assays [13, 16].

Unlike cells treated with cyclophosphamide or ifosfamide, treatment with paclitaxel resulted in cytotoxicity in the parental V79 cells. There was, however, less cell kill with CYP-expressing cells, suggesting metabolic inactivation of paclitaxel by enzymes expressed by these cells. In addition, cotreatment with either MTP or TAO resulted in a reduction in cell kill in CYP2B1- and CYP3A4-expressing cells, respectively. Initial studies had identified CY2C8 as the major enzyme in paclitaxel metabolism through the generation of 6-hydroxytaxol [18]. However, more recent studies have suggested that CYP3A4 catalytic activity is also important in overall paclitaxel metabolism in humans [15, 23]. The results of our study also suggest a potential role for CYP2B1 and CYP2E1 in paclitaxel metabolism, which requires further confirmation. Alternatively, CYP2B1 or CYP2E1 expression may modulate the cytotoxicity of paclitaxel by yet unknown mechanisms.

It is very likely that the differential cytotoxicity observed with these experiments was related to differences in the metabolism of cyclophosphamide, ifosfamide, and paclitaxel in these cell lines. This assumption is based on, firstly, the modulation of cytotoxicity by known competitive inhibitors of CYP. Secondly, a cytotoxic effect of oxazophosphorines was shown for culture incubates from CYP2B1- and CYP3A4-treated cells but not from parental V79 cells. The latter is due to a bystander

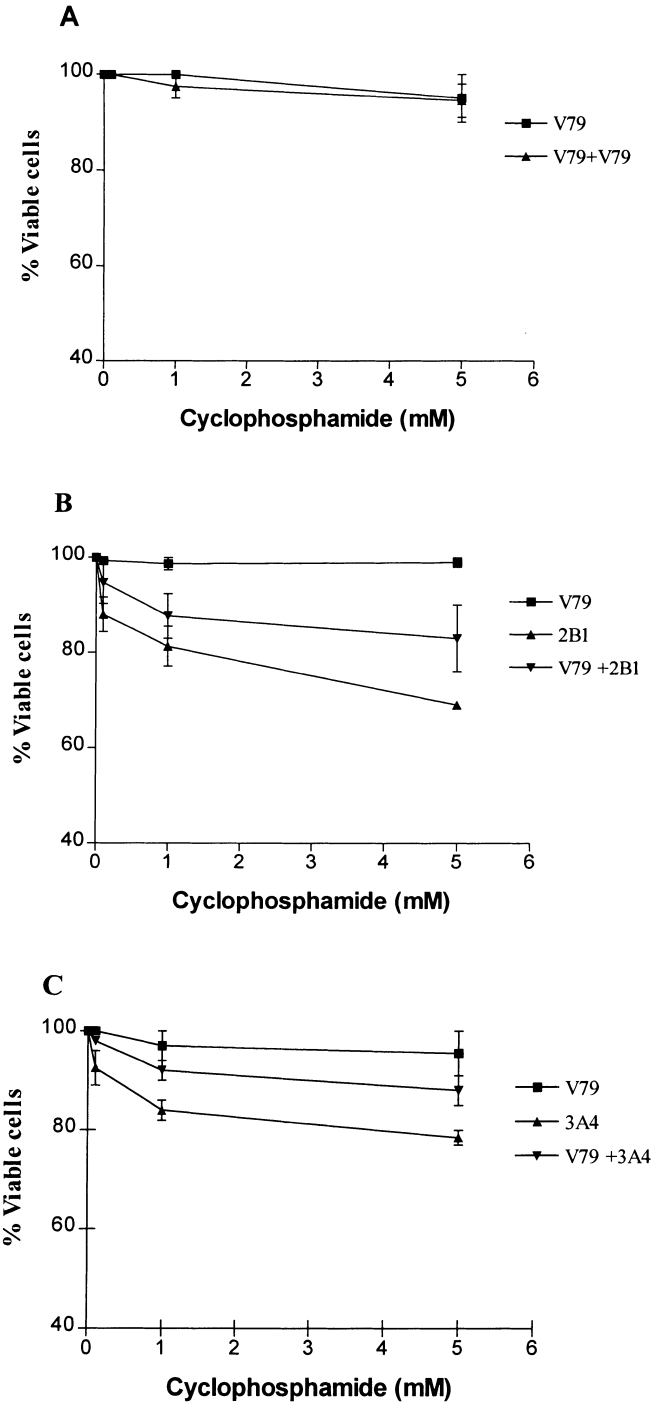


Fig. 5A–C The influence of cocultured CYP-expressing cells on the cyclophosphamide-induced cytotoxicity in nontransfected V79 cells. V79 cells were cocultured with either CYP2B1- (**A**) or CYP3A4- (**B**) expressing cells. In a control experiment cocultured V79-V79 cells were treated (**C**). The CYP2B1-, CYP3A4-, or non-CYP-expressing V79 cells were plated at a density of 100 000 per well. See Materials and methods for details of the experiment. The *y*-axis represents the percentage cell survival (MTT assay) relative to PBS-treated cells (*bars* ± SD). The values shown are means of two experiments. The results indicate that the presence of metabolically active cells increased the sensitivity of the parental V79 cells to cyclophosphamide. There was no change in sensitivity of these cells when cocultured with the nontransfected parental cells

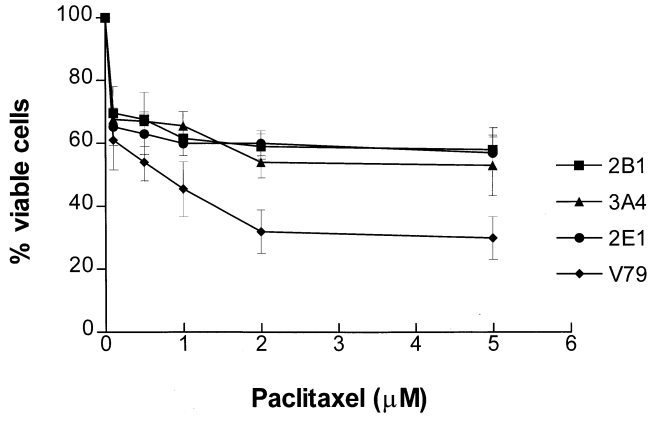


Fig. 6 Survival curves of parental and CYP-transfected V79 cells treated with paclitaxel. Cells were incubated with increasing concentrations of paclitaxel for 20 h. Cytotoxicity was determined using the MTT assay and the percentage of surviving cells relative to respective PBS-treated controls was calculated. Cell kill was demonstrated in cells expressing CYP2B1, CYP2E1, and CYP3A4. Transfected cells exhibited a lower cell kill compared to the parental V79 cells, suggesting inactivation of paclitaxel by the expressed CYP enzymes (*bars* ±SD)

cytotoxic effect which may be used in studying the sensitivity of tumor cells to drugs requiring metabolic activation by coculturing with metabolically active V79 cells.

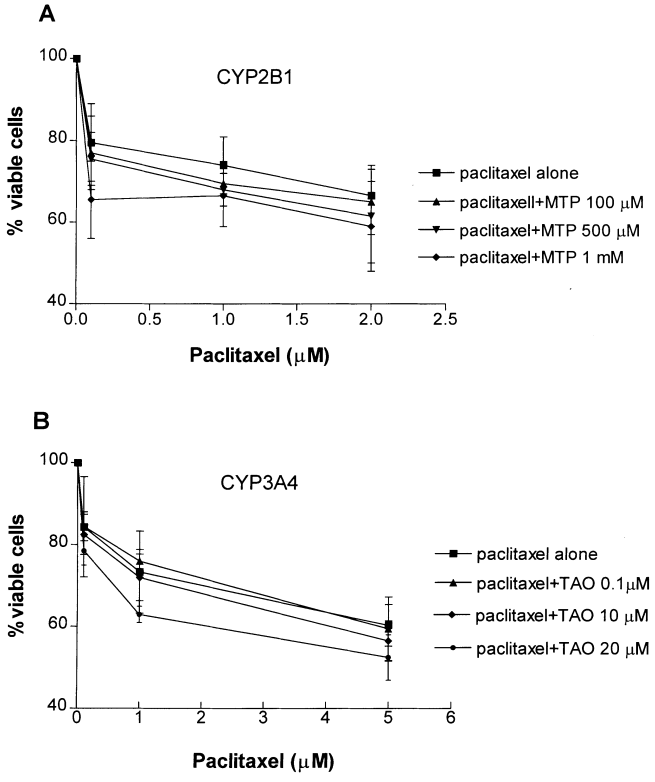


Fig. 7A,B The influence of coincubation with CYP substrates on the cytotoxicity induced by paclitaxel in V79 cells expressing either (**A**) CYP2B1 or (**B**) CYP3A4. The CYP substrates were simultaneously added with the paclitaxel followed by incubation for 20 h. Cell survival was based on the MTT assay and the percentage survival relative to PBS-treated cells was determined

Traditionally, human liver microsomes from several donors have been used, either individually or pooled, to avoid reliance on microsomes deficient in one or more metabolic pathways. Additionally, cofactors, primarily a redox-sustaining system, such as NADPH is required. V79 cells complement studying the metabolism of drugs using microsomes in several ways. The V79 cell system can confirm the apparent metabolic pathways determined by the microsomal incubations. Alternatively, expression systems such as the V79 cells may be used as an initial screening method for the determination of metabolic pathways that result in either activation or detoxification of cytotoxic drugs that would influence cell survival. The V79 expression system also complements the use of human hepatocyte cultures for studying drug metabolism because a major problem with hepatocyte cultures has been the scarcity of human liver samples and the marked interindividual variations in functional activities of isolated parenchymal cells [14]. V79 cells will also be very useful to test drug-drug interactions that would predict clinically significant drug interactions with cytotoxic therapy, especially with drugs that are subject to saturable metabolism. For example, nonlinear kinetics have been described for paclitaxel [22] and cyclophosphamide [4].

Coadministration of drugs with cytotoxic drugs that are metabolized by some CYPs may influence the cytotoxicity of these drugs, given their narrow therapeutic indices. For example, we have shown that the cotreatment of CYP3A4-expressing cells with midazolam, a sedative drug, reversed cyclophosphamide-induced cytotoxicity (Fig. 2). Clinical studies must ultimately determine the relevance of in vivo interactions between cyclophosphamide or ifosfamide and other drugs that are substrates of CYP3A. The availability of the different CYP transfectants may limit the use of these V79 cells in studying the metabolism of cytotoxic drugs. For example, CYP2C enzyme transfectants are lacking at this time. In addition, concentrations of drugs used to elicit cytotoxicity or even detectable levels of metabolites may not correspond to those expected in vivo.

In conclusion, V79 cells stably transfected with CYP enzymes are a potential tool for studying the metabolism of anticancer drugs when such metabolism results in the activation and/or deactivation of these drugs. This system would complement, and not replace, existing in vitro tools (e.g. human hepatocyte cultures). It would be of particular importance for studying cytotoxic drug behavior during early preclinical development, e.g. drug interactions, stereoselective drug metabolism. This system may help in the choice of appropriate probes for studying cytochrome P450 drug metabolism phenotypes in humans. Coculturing of tumor cells with a battery of genetically engineered single CYP-expressing cells may be a useful model to investigate enzyme-specific modulation of cytotoxicity of drugs requiring metabolic activation.

Acknowledgements J.D. gratefully acknowledges funding for the construction of the cell lines by the Bundesministerium fuer Bildung, Wissenschaft und Forschung (BMBF 0311259).

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