

Genetically Engineered V79 Chinese Hamster Cells Metabolically Activate the Cytostatic Drugs Cyclophosphamide and Ifosfamide

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V79 cells, genetically engineered to express active cytochromes P450IIB1 and P450IA1, were used to study the cytotoxicity and mutagenicity of cyclophosphamide and ifosfamide. Cyclophosphamide, tested up to a concentration of 2 mM, was not cytotoxic in V79 nor in the P450IA1-expressing V79-derived cell line XEM2. Pronounced cytotoxicity was, however, observed in the P450IIB1-expressing V79-derived cell line SD1. Induction of gene mutations (acquisition of 6-thioguanine resistance) was observed in SD1 cells as well, but the effects were weak. Ifosfamide was inactive in V79 cells, but was cytotoxic in SD1 cells. Ifosfamide mustard, an active metabolite of ifosfamide, was equally cytotoxic and showed similar mutagenic effects in SD1 and parental V79 cells. The results indicate that cyclophosphamide and ifosfamide are metabolically activated by cytochrome P450IIB1. In contrast, cytochrome P450IA1 was not capable of activating cyclophosphamide. Thus, V79-derived cell lines defined for their expression of a specific form of cytochrome P-450 can be used as diagnostic tools to identify the cytochrome P-450 that is responsible for the metabolic activation of drugs.

Introduction

V79 Chinese hamster cells have been used for mutagenicity testing for more than 10 years because they are easy to cultivate, grow rapidly, and have a stable karyotype with just one X-chromosome harboring the HPRT locus, which is convenient for mutagenicity studies. Chemicals are tested for their mutagenic potential by counting HPRT⁻ mutants surviving in medium containing 6-thioguanine. However, the situation gets complicated when the chemicals investigated are mutagenic only after metabolic activation because V79 do not express the cytochromes P-450. The cytochromes P-450 play a crucial role in the activation of most xenobiotics. The problem was circumvented by combining V79 cells with freshly prepared liver homogenate or liver cells, but this also created new problems. Externally generated metabolites might not be able to enter the V79 cells. They may react with macromolecules contained in the liver homogenate. For short-lived metabolites the diffusion pathways may be too long. In other cases the plasma membrane may act as a barrier. In any case, such metabolites would remain undetected and false negative results would be observed.

For this reason we have started a program employing gene technology to provide V79 cells with new metabolic capabilities related to xenobiotic metabolism. A V79-derived cell line expressing rat P450IIB1 (SD1) (1) and a V79-derived cell line expressing rat P450IA1 (XEM2) (2) have recently been established and characterized. Here, we demonstrate the application of these cell lines as diagnostic tools in drug metabolism studies.

Materials and Methods

Chemicals and Cell Culture

Cyclophosphamide, ifosfamide, and ifosfamide mustard were a gift of ASTA-Werke (Bielefeld, FRG).

V79 Chinese hamster cells and V79-derived cell lines SD1 and XEM2 were maintained in Dulbecco-Vogt's modified Eagle's medium (DMEM) supplemented with 5 to 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 µg/mL). For the SD1 and XEM2 stock cultures, the medium also contained 400 µg/mL G418.

Cytotoxicity and mutagenicity experiments were carried out as described previously (3). Briefly, 1.5×10^6 cells and 30 mL medium were seeded into a 15-cm Petri dish. After 18 hr, the test compound, dissolved in medium, was added. The exposure was terminated by a change of the medium 24 hr later. After an expression

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period of 6 days, with one subculture, the cells were counted and replated at a density of 10^6 per 15-cm Petri dish in medium containing 6-thioguanine (six replicate plates), or at a density of 100 cells per 6-cm Petri dish in medium without 6-thioguanine for the determination of cloning efficiency. The cultures were fixed and stained, and the colonies counted after about 7 days (cloning efficiency plates) or 10 days (selection plates). From these values, mutation frequencies were calculated.

Results

V79 cells survived in medium containing cyclophosphamide even at a concentration of 2 mM, whereas SD1 cells died in the presence of cyclophosphamide in a concentration-dependent manner, 98% of the cells being killed at an exposure concentration of 2 mM (Fig. 1). In medium containing ifosfamide, V79 cells survived, whereas SD1 cells died (Fig. 2). In contrast, ifosfamide mustard, an active metabolite of ifosfamide, showed similar cytotoxic activities in V79 and SD1 cells (Fig. 3).

The compounds were also investigated for the induction of gene mutations (Table 1). Cyclophosphamide and ifosfamide showed no effect in V79 cells, whereas cyclophosphamide weakly elevated the mutation frequency, and ifosfamide did not clearly elevate the mutation frequency in SD1 cells. These effects, which are

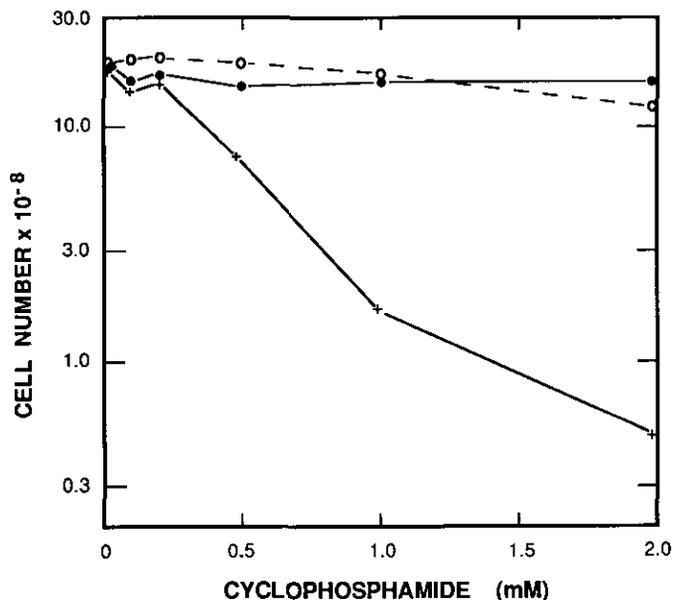


FIGURE 1. Surviving V79 (○), XEM2 (*), and SD1 (+) cells exposed to cyclophosphamide at various concentrations. Cells were exposed to cyclophosphamide for 24 hr and cultured for 5 additional days. In the middle of this expression period, the cultures were diluted to a density of 3×10^5 cells per plate. The figure shows the cell numbers at the end of the expression period, corrected for the dilution factor. Mutation frequencies, determined in the same experiment, are shown in Table 1.

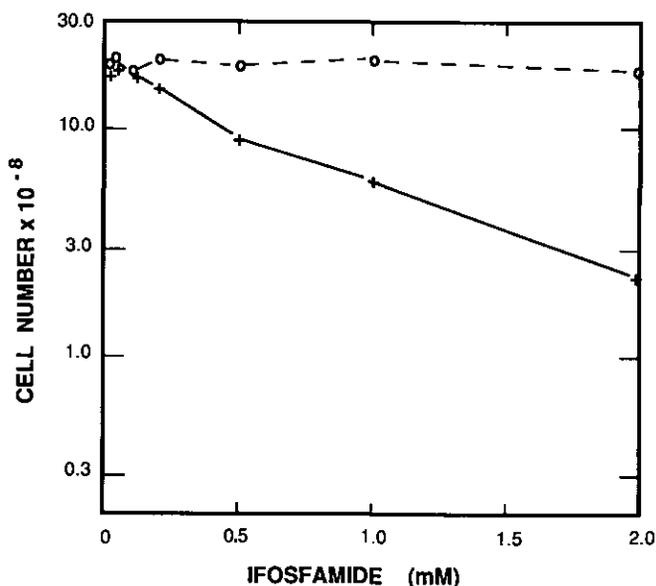


FIGURE 2. Surviving V79 (○) and SD1 (+) cells exposed to ifosfamide at various concentrations. The experimental conditions are described in the legend to Figure 1.

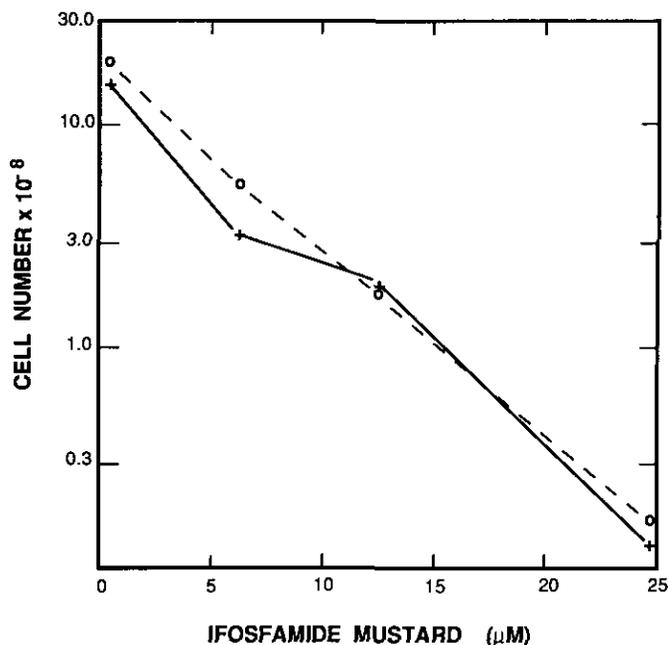


FIGURE 3. Surviving V79 (○) and SD1 (+) cells exposed to ifosfamide mustard at various concentrations. The experimental conditions are described in the legend to Figure 1.

just above the detection limit, have not been verified in repeat experiments, as cytotoxicity obviously was the more useful end point for studying the metabolic activation of these cytostatics. Ifosfamide mustard showed clear but weak mutagenicity in both SD1 and V79 cells.

Table 1. Mutagenicity of cyclophosphamide, ifosfamide, and ifosfamide mustard in V79 and SD1 cells.^a

Test compound	Concentration, μM	Mutation frequency $\times 10^6$	
		V79	SD1
Cyclophosphamide	0	0, 5	1, 3
	20	5	3
	50	4	6
	100	4	5
	200	7	12
	500	3	12
	1000	5	30
Ifosfamide	0	8, 11	3, 8
	50	6	3
	100	13	7
	200	12	4
	500	12	12
	1000	15	18
	2000	16	13
Ifosfamide mustard	0.0	5, 11	1, 2
	6.2	15	8
	12.3	66	21
	24.6	43	40

^a Acquisition of resistance to 6-thioguanine served as the marker for mutagenicity. Each value represents one treated culture and is based on six selection plates. The cytotoxicity data for the same experiment is shown in Figures 1–3.

Discussion

There are many indications in the literature that metabolic activation of cyclophosphamide by cytochrome P-450 is necessary for the generation of the mustard form, which is the penultimate cytotoxic compound. The observation that pretreatment of animals with phenobarbital increases the liver-enzyme-mediated bacterial mutagenicity of cyclophosphamide indicate that phenobarbital-inducible enzymes are involved in its activation (4). Using the V79-derived cell lines defined for the expression of one specific cytochrome P-450 form, we were able to show that cyclophosphamide is indeed activated by P450IIB1 expressed in the cell line SD1, the major phenobarbital-inducible cytochrome P-450. In contrast, no activation was observed with P450IA1 expressed in the cell line XEM2 (Fig. 1).

Ifosfamide is a structural isomer of cyclophosphamide. The differential cytotoxicity observed in this study demonstrates that this compound also requires metabolic activation and that P450IIB1 is capable of carrying it out. The finding that ifosfamide mustard showed very similar cytotoxic and mutagenic effects in V79 and SD1 cells confirms that the differences observed with the precursor ifosfamide are at the level of metabolic activation.

Cyclophosphamide and ifosfamide showed strong cytotoxicity, but only marginal mutagenicity, in SD1 cells. The same holds for ifosfamide mustard in SD1 as well as in V79 cells. These observations suggest that the induction of gene mutations is not a major mechanism for the cytotoxicity of these compounds, at least if the HPRT gene is considered representative.

Thus, it has been demonstrated that genetically engineered V79 cells expressing xenobiotic-metabolizing enzymes are a valuable tool for the evaluation of the metabolism and pharmacological action of drugs.

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