Cancer Chemother Pharmacol (1998) 41: 237–242

ORIGINAL ARTICLE

Michael J. Kelner · Trevor C. McMorris
Mark A. Montoya · Leita Estes · Slavomir F. Uglik
Mary Rutherford · Kyra M. Samson
Richard D. Bagnell · Raymond Taetle

Characterization of acylfulvene histiospecific toxicity in human tumor cell lines

Received: 26 March 1997 / Accepted: 14 July 1997

Abstract *Purpose*: Acylfulvene derivatives demonstrate marked efficacy in xenograft carcinoma models as compared with the parent illudin compounds. To elucidate the increased therapeutic efficacy of acylfulvene analogs, we compared them with the illudin compounds in terms of their in vitro cytotoxicity, cellular accumulation and DNA incorporation. *Methods*: The cytotoxicity of various acylfulvene analogs was tested in vitro against a variety of tumor cell lines. Radiolabelled acylfulvene analog was prepared and used for cellular accumulation and DNA incorporation studies. *Results*: The prototype acylfulvene analog retained selective histiospecific toxicity towards myeloid leukemia and various carcinoma cell lines. In vitro killing of tumor cells by acylfulvene required up to a 30-fold increase in molecules per cell, as compared with illudin S, indicating that acylfulvene was less toxic on a cellular level. At equitoxic concentrations, acylfulvene incorporation into genomic tumor cell DNA was equivalent to illudin S suggesting that cellular metabolism has a role in acylfulvene cytotoxicity. Analysis of cellular accumulation of acylfulvene into tumor cells revealed a markedly higher V_{max} for tumor cells, and a lower V_d for diffusion accumulation into other cells. *Conclusions*: The combination of higher V_{max} and lower V_d may explain the increased in vivo efficacy of acylfulvene.

M.J. Kelner (✉) · M.A. Montoya · L. Estes · S.F. Uglik
M. Rutherford · K.M. Samson · R.D. Bagnell
Department of Pathology 8320, UCSD Medical Center,
200 West Arbor Drive, San Diego, CA 92103, USA
Tel. +619-543-5976; Fax +619-543-3730
email mkelner@ucsd.edu

T.C. McMorris
Department of Chemistry, University of California,
San Diego, La Jolla, CA 92093, USA

R. Taetle
Department of Hematology, Arizona Cancer Center,
1501 North Campbell Avenue, University of Arizona,
Tucson, AZ 85724, USA

Key words Illudins · Acylfulvene · Chemotherapeutic · HMAF

Introduction

Illudins are sesquiterpene compounds derived from the mushroom *Omphalotus illudens* and related species of basidiomycetes [1–6], whose chemical structure is different from known chemotherapeutic agents [4]. Using short (2-h) in vitro exposures, illudins are preferentially cytotoxic to a variety of hematopoietic and solid tumor cells at nano- to picomolar concentrations [7, 8]. In contrast, normal bone marrow progenitors or fibroblasts are relatively resistant to illudins and require exposures to micromolar concentrations for equivalent in vitro cytotoxicity [7, 8]. The preferential illudin cytotoxicity stems from differences in intracellular accumulation of the drug resulting from an energy-dependent uptake process absent in most nontumor cells [8]. In contrast to DNA damage produced by many other anticancer drugs, the repair of illudin-induced DNA damage requires early action of the ERCC2 and ERCC3 DNA helicases before repair can proceed [9].

Although Illudin S has proved too toxic for effective use in vivo [7, 10], we have synthesized analogs more efficacious against in vivo experimental tumors than the parent compound. The first group (dehydroilludin M) increases the lifespan in a human lung carcinoma xenograft model resistant to ten conventional chemotherapeutic agents including paclitaxel [10, 11]. The second group of analogs (acylfulvenes) are even more effective in the lung xenograft model [12]. One acylfulvene analog, 6-hydroxymethylacylfulvene, has demonstrated marked efficacy in the MV522 lung carcinoma, MX1 breast carcinoma, KB epidermoid carcinoma, and HT29 colon carcinoma xenograft models [13, 14], and is currently in phase I chemotherapeutic clinical trials.

Acylfulvene analogs possess other desirable characteristics as cancer chemotherapeutic agents. Multidrug-

resistant cell lines, resistant by different mechanisms, remain sensitive in vitro to acylfulvene analogs [12]. DNA helicase-deficient cells remain sensitive to the acylfulvenes indicating that repair of acylfulvene-induced DNA damage requires action of ERCC2 and ERCC3 DNA helicases before repair can proceed [12]. In contrast, repair of DNA damage produced by other anticancer agents does not require the action of these DNA-repair helicases [9].

For the above reasons, we chose an acylfulvene analog for detailed in vitro cytotoxicity studies on the basis that increased acylfulvene in vivo efficacy (versus illudins) is unclear. The increased antitumor efficacy of acylfulvenes may arise from differences in energy-dependent accumulation in tumor cells, differences in intracellular metabolism, or differences in DNA damage or repair. We present evidence that in vivo efficacy of acylfulvenes is likely related to preferential energy-dependent accumulation of illudins in tumor cells.

Materials and methods

Cell lines

The following cell lines were maintained in either RPMI-1640 or Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah) as previously described [7, 8]: human myeloid leukemia cell line HL60 [15]; human B-cell-derived leukemia/lymphoma cell line 8392 [16]; human lung adenocarcinoma cell line MV522 [17]; colon adenocarcinoma lines HT29 [18] and SW48 [19]; and human breast carcinoma cell lines MCF7 [20], MDA231 [21] and MDA468 [22]. The CHO AA8 line was obtained from Dr. Larry Thompson [23]. All cell lines were routinely screened for mycoplasma. The cytotoxic effects of acylfulvene in vitro were assayed by either viable cell counts, inhibition of thymidine incorporation into DNA, or by clonogenic colony-forming assays (CFA) as previously described [7, 8].

Preparation of illudin analogs and radiolabelled acylfulvene

Omphalotus illudens subtype S.B. Carey 4435 (formerly *Clitocybe illudins*) was obtained from the New York Botanical Garden (New York, N.Y.). Illudin S and illudin M were prepared from cultures as previously described [1–3]. Dehydroilludin M was prepared as previously described by oxidation of the parent component illudin M with pyridinum dichromate in methylene chloride [4]. Acylfulvene and acylfulvene dimer were prepared from the parent compound illudin S by reaction with 2 *M* H_2SO_4 [24, 25]. Bromofulvene was prepared by reacting acylfulvene with *N*-bromosuccinimide in acetonitrile and iodofulvene was prepared by reacting the acylfulvene with iodine in a solution of methylene chloride with silver trifluoroacetate [24]. The chemical structures of the analogs are shown in Fig. 1. Radiolabelled illudin S was prepared by the addition to the fermentation broth of the precursor tritiated sodium acetate [9]. The radiolabelled illudin S was converted to radiolabelled acylfulvene as previously described [12]. The specific activity of the radiolabelled acylfulvene used in this study was 320 mCi/mmol.

Illudin cellular and genomic DNA accumulation studies

Uptake of the tritiated acylfulvene into tumor cells was performed as previously described [8, 9, 26]. Incorporation of tritiated acylfulvene into genomic DNA was detected as previously described using a GNOME DNA Isolation Kit (BIO-101, Vista, Calif.) [9].

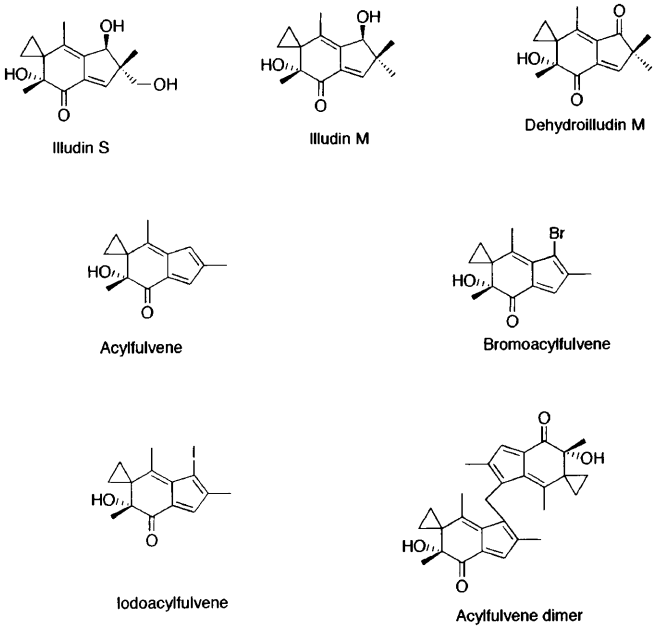

Fig. 1 Chemical structures of the illudin derivatives studied

Statistics

Statistical analysis of data included linear regression (least squares), correlation coefficients, and the nonparametric Mann-Whitney analysis for comparing acylfulvene with illudin S. Analysis was performed using GraphPad Instat Software (version 2.02) (La Jolla, Calif.).

Results

Cytotoxicity studies

Although acylfulvene derivatives are markedly efficacious in the MV522 xenograft model [12], they were less toxic in vitro than illudin S in both the HL60 and MV522 target cell lines with a 2-h exposure period (Table 1). The prototype fulvene analog, acylfulvene, was markedly less active against 8392 B-cell line (Table 1) using a 2-h exposure. As this compound has a short in vivo half-life in animals of approximately 8 min [27, 28], we studied the cytotoxicity of this derivative against the cell lines using a 15-min exposure and CFAs. Using these conditions acylfulvene was markedly less toxic to MV522 and HL60 cells than illudin S (Fig. 2). However, the relative selective in vitro cytotoxicity observed with other illudins remained, as acylfulvene was relatively nontoxic to the 8392 B-cell tumor cell lines even at high micromolar concentrations (Fig. 2).

Various cell lines have been previously noted to be comparatively sensitive to illudin S after 48 h [7, 8], but marked variation in cell line sensitivity has been noted with a 2-h exposure [7, 8]. Again, acylfulvene retained this pattern. The cell lines tested were sensitive to acylfulvene using 48-h exposure (Table 2), but there was

Table 1 Cytotoxicity of illudin analogs in the screening cell lines. The 2-h exposure screening assays were performed using inhibition of thymidine incorporation into genomic DNA (see Methods). Values are the means ± standard deviation of three to five experiments

Analog	Exposure time (h)	Cell line HL60 (n*M*)	8392 (n*M*)	MV522 (n*M*)
Illudin S	2	10 ± 1	236 ± 31	19 ± 6
Dehydroilludin M	2	377 ± 81	36 000 ± 4000	1 830 ± 380
Acylfulvene	2	998 ± 244	66 000 ± 13 000	1 200 ± 100
Acylfulvene dimer	2	24 000 ± 4000	46 000 ± 1400	10 500 ± 1500
Iodofulvene	2	2 600 ± 300	10 300 ± 500	1 000 ± 150
Bromofulvene	2	800 ± 100	17 200 ± 900	4 200 ± 400

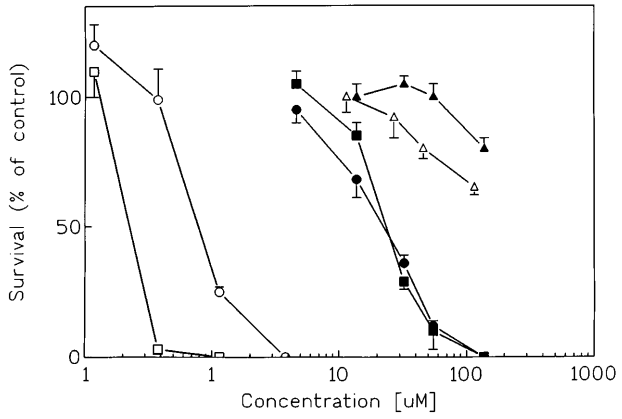

Fig. 2 Cytotoxicity of acylfulvene compared with illudin S in human tumor cell lines with a 15-min exposure, as determined by a colony-forming assay (□ illudin S toxicity in HL60 myeloid leukemia cell lines, ○ MV522 lung carcinoma, △ 8392 B-cells; ■ acylfulvene toxicity in HL60 myeloid leukemia cell lines, ● MV522 lung carcinoma, ▲ 8392 B-cells)

marked variation in acylfulvene sensitivity with a 2-h exposure.

The IC_{50} values obtained using CFAs were correlated ($r = 0.9977$, $P < 0.001$) with values obtained from the rapid thymidine inhibition incorporation assay (Table 2). Cell lines previously noted to display energy-dependent cellular accumulation of illudin S were more sensitive to the acylfulvene analog using a 2-h exposure. In contrast, the 8392 B-cell line, previously shown to lack energy-dependent accumulation of illudin S [8], was resistant to acylfulvene with a short 2-h exposure (Table 2).

Table 2 Cytotoxicity of acylfulvene with short (2 h) and prolonged exposure (48 h) in various tumor cell lines. The values are the mean (±SD) IC_{50} values (n*M*) from three to five experiments. (*Td* thymidine inhibition assay, *CFA* colony-forming assay, *TB* trypan blue exclusion assay (see Methods), *NT* not tested)

	2-h Td	2-h CFA	48-h TB
HL60	988 ± 244	2 100 ± 100	364 ± 74
SW48	4 900 ± 1100	5 500 ± 900	590 ± 40
HT29	460 ± 20	1 800 ± 100	760 ± 20
MCF7	4 700 ± 700	1 000 ± 160	200 ± 20
MDA231	1 300 ± 200	900 ± 100	70 ± 20
MDA468	600 ± 20	NT	660 ± 60
MV522	1 200 ± 100	2 200 ± 100	350 ± 20
8392	66 000 ± 13000	81 900 ± 2700	830 ± 150

Table 3 Intracellular accumulation of acylfulvene

	Rate[a] (pmol/h)	2-h total[b] (pmol)
HL60	17 ± 2	24 ± 1
MV522	201 ± 10	147 ± 8
SW48	16 ± 2	17 ± 1
HT29	274 ± 30	190 ± 10
8392	3.7 ± 0.5	7.4 ± 1.0

[a] Initial rate of acylfulvene intracellular accumulation per 10 million cells when exposed to 462 n*M* (100 μg/ml) acylfulvene for 15 min ($n = 3$, mean ± SD)
[b] Amount of acylfulvene (pmol) accumulated intracellularly in 10 million cells with a 2-h exposure to 462 n*M* (100 μg/ml) of acylfulvene ($n = 3$, mean ± SD)

Cellular uptake and incorporation into genomic DNA

Tumor cells were incubated with acylfulvene at the low concentration of 462 n*M* (100 ng/ml) and the initial intracellular accumulation rate and total cellular accumulation determined (Table 3). The number of acylfulvene molecules required to kill 50% of cells (as determined using the CFA) after a 2-h exposure was determined to compare the results with those of prior studies with illudin S. Despite a several log variation in the in vitro IC_{50} value (Table 2), the estimated number of molecules of acylfulvene required to kill a cell appeared relatively constant, with the possible exception of the HL60 myeloid line (Table 4). The number of acylfulvene molecules required to kill a specific tumor cell line, however, was significantly more (Mann-Whitney $P < 0.01$) than the number of molecules of the parent illudin S compound required (Table 4). The amounts of acylfulvene incorporated into genomic DNA at the respective IC_{50} values were generally similar in the various cell lines (Table 5); the exception was the relatively resistant 8392 B-cell line which required a fivefold higher incorporation of acylfulvene for equivalent killing.

Characterization of acylfulvene energy-dependent cellular accumulation

The acylfulvene energy-dependent cellular accumulation into the HL60 and MV522 tumor cells was assessed and compared with that of illudin S. Acylfulvene uptake into HL60 cells increased with time and was retarded by the presence of the metabolic inhibitors, 2-deoxyglucose and

Table 4 Number of acylfulvene molecules required to kill 50% of cells as determined using a CFA with a 2-h exposure

Cell line	Acylfulvene (no. of molecules/cell)	Illudin S[a] (no. of molecules/cell)
HL60	3 000 000 ± 420 000[b]	78 000 ± 12 000
MV522	29 700 000 ± 600 000	1 720 000 ± 70 000
8392	24 600 000 ± 4 400 000	852 000 ± 108 000
SW48	13 900 000 ± 300 000	452 000 ± 30 000
HT29	39 200 000 ± 1 100 000	480 000 ± 18 000
MDA231	8 700 000 ± 300 000	516 000 ± 18 000
MCF7	7 700 000 ± 800 000	1 140 000 ± 36 000
AA8	8 900 000 ± 200 000	751 000 ± 5800

[a] The illudin S values are as previously reported [30] listed here to allow a direct comparison with acylfulvene
[b] Refers to the mean ± standard deviation ($n = 3$)

Table 5 Incorporation of acylfulvene into DNA or RNA after 2 h

Cell line	IC_{50}[a] (nM/l)	DNA incorporation (femtomoles/μg)	RNA incorporation (femtomoles/μg)
HL60	443	17.3 ± 0.8	12.1 ± 0.2
MV522	477	21.1 ± 0.7	11.9 ± 0.3
MDA231	190	10.5 ± 1.7	
MCF7	212	15.0 ± 0.5	
SW48	1 180	16.0 ± 0.8	
HT29	379	19.3 ± 1.3	
8392	17 670	85 ± 25	

[a] IC_{50} refers to the acylfulvene concentration at which 50% killing occurs by CFA for that cell line, and is the concentration to which that cell line was exposed for 2 h, before determining incorporation of acylfulvene into DNA or RNA

antimycin A (Fig. 3), similar to results previously obtained with illudin S [8]. Acylfulvene accumulation in MV522 cells, however, was markedly increased as compared with that of illudin S ($P < 0.01$), and was also retarded by the presence of metabolic inhibitors (Fig. 3).

Energy-dependent uptake of acylfulvene into both MV522 and HL60 cells was concentration-dependent at low external concentrations, but saturated at high concentrations (Fig. 4). The energy-dependent acylfulvene cellular uptake V_{max} and K_m values in HL60 myeloid

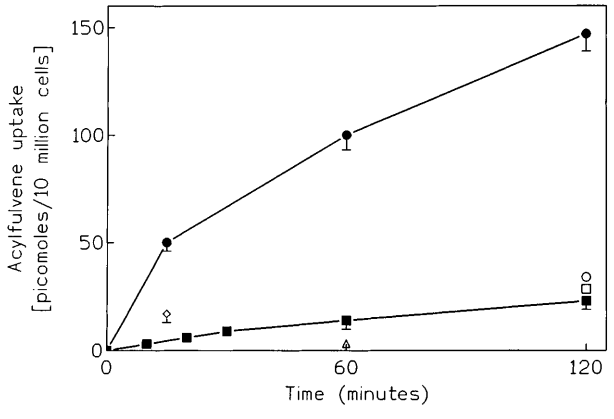

Fig. 3 Energy-dependent cellular accumulation of acylfulvene as a function of time in HL60 myeloid leukemia (■) and MV522 lung carcinoma cells (●). The isolated points represented by an *open triangle* and an *open diamond* indicate acylfulvene uptake in the presence of the metabolic inhibitors 2-deoxyglucose and antimycin A into HL60 and MV522 cells, respectively. The isolated points represented by an *open square* and an *open circle* indicate illudin S uptake into HL60 and MV522 cells, respectively

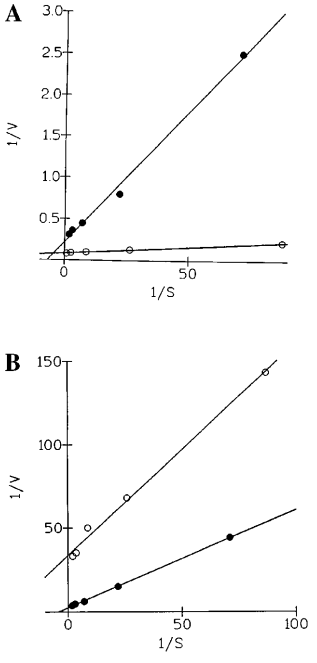

Fig. 4A,B Comparison of Michaelis-Menten analysis for illudin S (○) and acylfulvene (●) energy-dependent cellular accumulation in (**A**) HL60 myeloid leukemia cells and (**B**) in MV522 lung carcinoma cells

Table 6 Analysis of acylfulvene cellular uptake

Cell line	Parameter	Acylfulvene		Illudin S	
		Michaelis-Menton (linear)	Algorithm[a] (nonlinear)	Michaelis-Menton (linear)	Algorithm[a] (nonlinear)
HL60	V_{max}	3.6 ± 0.2[b]	3.6 ± 0.5	27.8 ± 2.0	29.0 ± 5.1[b]
	K_m	7.8 ± 1.5	7.8 ± 3.2	7.1 ± 1.5	7.1 ± 1.6
	V_d	0.018		0.34	
MV522	V_{max}	348 ± 19	333 ± 41	33.6 ± 1.6	34.8 ± 3.0
	K_m	18.1 ± 2.6	16.2 ± 4.8	5.6 ± 0.9	6.0 ± 1.4
	V_d	0.013		0.13	
8392	V_{max}	ND[c]	4.04 ± 1.7[d]	6.0 ± 2.6	3.0 ± 0.9
	K_m	ND	73 ± 5	81.0 ± 5.0	25 ± 2
	V_d	0.010		0.05	

[a] Analysis of data using nonlinear regression algorithm was performed using the Kinetics software [29]
[b] Vm in picomoles/minute for 10 million cells; K_m in μM; V_d in min^{-1} for 10 million cells when S expressed in μM
[c] Linear Michaelis-Menton analysis did not yield an answer for the 8392 B-cell line (see text)
[d] Analysis of 8392 energy-dependent cellular accumulation required a 1-h exposure in contrast to the 15 min required for the HL60 and MV522 cell lines

cells were similar to those previously reported for illudin S (Table 6). The K_m value for acylfulvene in MV522 cells was also similar to that previously reported for illudin S (Table 6). The V_{max} for acylfulvene in MV522 cells, however, was markedly higher than that for illudin S (Table 6). We were unable to detect evidence of energy-dependent cellular accumulation in the 8392 cells using a 15-min exposure. With a 1-h exposure, however, there was low level energy-dependent cellular accumulation with a relatively high K_m value when analyzed by a nonlinear algorithm method.[1] The V_{max} and K_m values for acylfulvene in 8392 cells were similar to those of illudin S [8], but the V_d value was fivefold lower.

Discussion

The prototype fulvene analog, acylfulvene (Fig. 1), has been previously noted to be effective in a metastatic xenograft lung carcinoma model resistant to treatment with ten conventional chemotherapeutic agents [12]. In addition, acylfulvene retains promising features of illudins, such as the ability to kill multidrug-resistant cells resistant by a variety of mechanisms. DNA helicase-deficient cells are also sensitive to acylfulvene as previously demonstrated for illudin S. These DNA helicase-deficient cells do not display increased sensitivity to other chemotherapeutic agents, suggesting that acylfulvene produces an unusual DNA damage also noted with illudins [9].

The present studies showed that acylfulvene retained the selective in vitro cytotoxicity towards myeloid leukemia, breast, colon, and lung carcinoma cells using short exposure periods (Fig. 2). As with other illudins, with a more prolonged exposure, acylfulvene was equally toxic to all cell lines tested (Table 2). Prior studies have shown that prolonged exposure allows illudins to passively diffuse across cell membranes, while the selective in vitro cytotoxicity using short exposure times correlates with energy-dependent illudin accumulation [8, 30].

In vitro killing of tumor cells by acylfulvene required up to a 70-fold increase in molecules per cell compared with illudin S (Table 4), indicating that on a cellular level, acylfulvene is less toxic than the parent compound illudin S (nonparametric Mann-Whitney test, $P < 0.01$). At equitoxic concentrations, however, the incorporation of acylfulvene into tumor cell genomic DNA (Table 5) was equivalent ($P > 0.20$) to the incorporation of illudin S [30]. The relatively high number of acylfulvene molecules required, combined with the equivalent genomic DNA incorporation, indicates that cellular metabolism is likely to play a role in the reduced in vitro cytotoxicity of acylfulvene.

Analysis of cellular accumulation of acylfulvene into MV522 tumor cells (Figs. 3 and 4) revealed a markedly higher V_{max} for energy-dependent accumulation of acylfulvene compared with that of illudin S (Table 6). In a cell line lacking the energy-dependent process, the 8392 B-cell line, there was a markedly lower V_d coefficient. Together these two findings may explain the increased in vivo efficacy of acylfulvene compared with illudin S. The higher V_{max} allows increased accumulation of acylfulvene into tumor cells at low plasma concentrations. The markedly higher K_m and relatively lower V_d coefficient for the acylfulvene will result in a reduction in the number of molecules either actively (energy-dependent) or passively entering nontumor cells, explaining the decrease in nonspecific in vivo toxicity of acylfulvene (as compared with illudin S).

The effect of these alterations can best be observed by comparing the cellular accumulation of acylfulvene and

[1] Linear transformation of the Michaelis-Menten equation does not weight all data points equally, and often leads to erroneous determination of kinetic parameters. The use of nonlinear regression algorithms which fit data directly to the equation, without prior weighting of data points, eliminates this problem. For further discussion see reference 29.

illudin S in relatively sensitive and insensitive cells. Cellular accumulation follows the standard formula:

$$V = \frac{(V_{max} \times S)}{(K_m + S)} + [V_d \times S]$$

Administering a therapeutic dose of acylfulvene of 5 mg/kg to dogs by a 15-min intravenous infusion results in a steady-state plasma concentration of 5 μM [27, 28] during the infusion. Under these conditions the 8392 B-cells (nontarget) would accumulate acylfulvene at 0.3 pmol/10 million cells per minute whereas MV522 cells (target) accumulate the drug at 79 pmol/10 million cells per minute, a 263-fold increase. In contrast, at a 5 μM plasma concentration of illudin S, relatively resistant 8392 B-cells would accumulate the toxin at 0.8 pmol/10 million cells per minute, whereas MV522 cells would accumulate illudin S at 10.3 pmol/10 million cells per minute, or only a 13-fold difference. Thus, pharmacologic concentrations of acylfulvene permit preferential accumulation into tumor cells compared with the natural product precursor illudin S toxin.

Acknowledgements Supported by funds provided by the Cigarette and Tobacco Tax Fund of the State of California through the Tobacco-related Diseases Research Program of the University of California (Award 4RT-0226 and 4IT-0194), and by funds provided by MGI PHARMA, Inc., Minneapolis, Minnesota.

References

1. Anchel M, Hervey A, Robbins WJ (1950) Antibiotic substances from Basidiomycetes. VII. *Clitocybe illudins*. Proc Natl Acad Sci USA 36: 30–36
2. McMorris TC, Anchel M (1963) The structures of the Basidiomycetes metabolites illudin S and illudin M. J Am Chem Soc 85: 831–832
3. McMorris TC, Anchel M (1965) Fungal metabolites. The structures of the novel sesquiterpenoids illudin-S and -M. J Am Chem Soc 87(7): 1594–1600
4. McMorris TC, Kelner MJ, Wang W, Estes LA, Montoya MA, Taetle R (1992) Structure-activity relationships of illudins: analogs with improved therapeutic index. J Org Chem 57: 6876–6883
5. McMorris TC, Kelner MJ, Chadha RK, Siegal JS, Moon S, Moya MM (1989) Structure and reactivity of illudins. Tetrahedron 45: 5433–5440
6. McMorris TC, Kelner MJ, Wang W, Moon S, Taetle R (1990) On the mechanism of toxicity of illudins. Chem Res Toxicol 3: 574–579
7. Kelner MJ, McMorris TC, Beck WT, Zamora JM, Taetle R (1987) Preclinical evaluations of illudins as anticancer agents. Cancer Res 47: 3186–3189
8. Kelner MJ, McMorris TC, Taetle R (1990) Preclinical evaluation of illudins as anticancer agents: basis for selective cytotoxicity. J Natl Cancer Inst 82: 1562–1565
9. Kelner MJ, McMorris TC, Estes L, Rutherford M, Montoya M, Goldstein J, Samson K, Starr R, Taetle R (1994) Characterization of illudin S sensitivity in DNA repair-deficient Chinese hamster cells. Biochem Pharmacol 48: 403–409
10. Kelner MJ, McMorris TC, Taetle R (1995) In vitro and in vivo studies on the anticancer activity of dehydroilludin M. Anticancer Res 15: 873–878
11. Kelner MJ, McMorris TC, Estes L, Starr R, Samson K, Varki N, Taetle R (1995) Nonresponsiveness of the metastatic human lung carcinoma MV522 xenograft to conventional anticancer agents. Anticancer Res 15: 867–872
12. Kelner MJ, McMorris TC, Estes L, Starr RJ, Rutherford M, Montoya M, Samson K, Taetle R (1995) Efficacy of acylfulvene illudin analogs against a metastatic lung carcinoma MV522 xenograft nonresponsive to traditional anti-cancer agents: retention of activity against various mdr phenotypes and unusual cytotoxicity against ERCC2 and ERCC3 DNA helicase-deficient cells. Cancer Res 55: 4936–4940
13. Kelner MJ, McMorris TC, Estes L, Wang W, Samson KM, Taetle R (1996) Efficacy of HMAF (MGI-114-02) in the MV522 metastatic lung carcinoma xenograft model nonresponsive to traditional anticancer agents. Invest New Drugs 14: 161–167
14. MacDonald JR, Muscoplat CC, Dexter DL, Mangold GL, Chen S-F, Kelner MJ, McMorris TC, Von Hoff DD (1997) Preclinical antitumor activity of 6-hydroxymethylacylfulvene, a semisynthetic derivative of the mushroom toxin illudin S. Cancer Res 57: 279–283
15. Collins SJ, Gallo RC, Gallagher RE (1977) Continuous growth and differentiation of human myeloid leukeaemic cells in suspension culture. Nature 270: 347–349
16. Leonard JE, Taetle R, To D, Rhyner K (1985) Preclinical studies on the use of selective antibody ricin conjugated in autologous bone marrow transplantation. Blood 65: 1149
17. Varki NM, Roome L, Sparkes RS, Miller JE (1987) Microscopic metastasis of a human lung carcinoma cell line in athymic nude mice: isolation of a metastatic variant. Int J Cancer 40: 46–52
18. Leibovitz A, Stinson JC, McCombs WB 3rd, McCoy CE, Mazur KC, Mabry ND (1976) Classification of human colorectal adenocarcinoma cell lines. Cancer Res 36: 4562–4569
19. Fogh J, Trempe G (1975) New human tumor cell lines. In: Fogh J (ed) Human tumor cells in vitro. Plenum Press, New York London, pp 115–159
20. Cowan KH, Batist G, Tulpule A, Sinha BK, Myers CE (1986) Similar biochemical changes associated with multi-drug resistance in human breast cancer cells and carcinogen-induced resistance to xenobiotics in rats. Proc Natl Acad Sci USA 83: 9328–9332
21. Fuqua SAW, Moretti-Rojas IM, Schneider SL, McGuire WL (1987) P-glycoprotein expression in human breast cancer cells. Cancer Res 47: 2103–2106
22. Cailleau R, Olive M, Cruciger QV (1978) Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro 14: 911–915
23. Thompson LH, Rubin JS, Cleaver JE, Whitmore GF, Brookman K (1980) A screening method for isolating DNA-repair deficient mutants of CHO cells. Somat Cell Genet 6: 391–405
24. McMorris TC, Kelner MJ, Wang W, Diaz MA, Estes LA, Taetle R (1996) Acylfulvenes, a new class of potent antitumor agents. Experientia 52: 75–80
25. Weinreb S, McMorris TC, Anchel M (1971) Fulvenes derived from illudin S. Tetrahedron Lett 38: 3489–3491
26. Kelner MJ, Bagnell R (1989) Paraquat resistance associated with reduced NADPH reductase in an energy-dependent paraquat-accumulating cell line. Arch Biochem Biophys 274: 366–374
27. Brandsteterova E, Kelner MJ, McMorris TC, Estes L, Bagnell R, Montoya A (1992) HPLC determination of a new anticancer agent (acylfulvene) in serum. Neoplasma 39: 369
28. Brandsteterova E, Kelner MJ, McMorris TC, Wang W, Bagnell R (1993) HPLC analysis of novel anticancer agents – illudins and their analogs. J Liquid Chromatogr 16: 115–126
29. Brooks SPJ (1992) A simple computer program with statistical tests for the analysis of kinetics. Biotechniques 13: 906–911
30. Kelner MJ, McMorris TC, Montoya MA, Estes L, Rutherford M, Samson KM, Taetle R (1997) Characterization of cellular accumulation and toxicity of illudin S in sensitive and non-sensitive tumor cells. Cancer Chemother Pharmacol 40: 65–71