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Characterization of cellular accumulation and toxicity of illudin S in sensitive and nonsensitive tumor cells

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Abstract Illudins are novel low molecular weight natural products cytotoxic to human tumor cells in vitro. Illudin-derived analogs are effective against experimental human cancers nonresponsive to conventional anticancer agents. It is not known why some illudin analogs are more efficacious in vitro and in vivo than other analogs. Therefore, the in vitro cytotoxicity of the parent compound illudin S towards tumor cells was characterized using radiolabeled drug. Two cell lines sensitive at nanomolar concentrations using only a 15-min exposure period displayed a saturable, energy-dependent accumulation of illudins with relatively low K_m and high V_{max} values. A nonsensitive cell line, requiring millimolar concentrations to achieve in vitro toxicity, showed minimal illudin uptake with higher K_m and lower V_{max} values. No release of radioactivity could be demonstrated from tumor cells, indicating that there was no efflux of illudin S (or metabolites) from these cells. The number of intracellular illudin S molecules required to kill 50% of cells of different tumor cell lines varied from 78 000 to 1 114 000 molecules per cell and was correlated with the 2-h IC_{50} value determined using a colony-forming assay. Illudin S was cytotoxic to a variety of multidrug-resistant tumor cell lines regardless of whether resistance was mediated by gp170/mdrl, gp180/MRP, GSHTR-pi, topoisomerase I, topoisomerase II, increased DNA repair capacity, or alterations in intracellular thiol content. Information obtained in this study could be used to design clinical phase I trials and to develop analogs with improved therapeutic indexes.

Key words Illudin · Chemotherapeutic · Multidrug resistance · *Omphalotus illudens*

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Introduction

Illudins are sesquiterpene compounds derived from the mushroom *Omphalotus illudens* and related species of basidiomycetes [1, 2]. The chemical structure of illudin differs from known chemotherapeutic agents [3]. The illudins are preferentially cytotoxic using a short in vitro exposure (less than 2 h) to a variety of hematopoietic leukemia and solid tumor cells at picomolar to nanomolar concentrations [4, 5]. Solid tumors sensitive to illudins in vitro include breast, lung, colon and ovarian carcinomas. In contrast, normal bone marrow progenitors or fibroblasts are relatively resistant to illudins and require exposure to micro- or millimolar concentrations for cytotoxicity [4–8].

The illudins possess other characteristics desirable in chemotherapeutic agents. A variety of multidrug resistant (mdr) tumor cell lines remain sensitive to the illudins [4, 9]. Repair of illudin-induced DNA damage, in contrast to damage induced by other known chemotherapeutic agents (such as mitomycin C and cisplatin), requires the action of ERCC2/XPD and ERCC3/XPB DNA helicase repair enzymes [10]. The ERCC2/XPD and ERCC2/XPB DNA repair helicases are also components of the TFIIH transcription complex, which may explain why illudins preferentially inhibit DNA synthesis within minutes of entering a cell [4, 9]. Preferential cytotoxicity of illudins for sensitive tumor cells appears to result from rapid intracellular accumulation

of toxin by an energy-dependent process absent from nonsensitive cells [5].

Although the therapeutic index of parent illudin compounds has proved too low for use in tumor-bearing animals [9], we have synthesized analogs which demonstrate greater in vivo antitumor effects. The dehydroilludin analogs increase the lifespan of mice bearing a human lung carcinoma MV522 xenograft model resistant to ten conventional chemotherapeutic agents including taxol [9, 11]. A different class of illudin-derived analogs, called acylfulvenes, are markedly effective at prolonging lifespan in the MV522 model [12].

The basis for increased activity of the illudin analogs over the parent compounds is unclear. Increased antitumor efficacy might arise from more favorable pharmacokinetics, tissue distribution or uptake, variations in cellular transport or metabolism, or difficulty in repair of the drug-induced DNA damage. To help determine relevant mechanism(s), we further characterized the relationships between illudin S uptake in vitro and relative toxicity.

Materials and methods

Cell culture

The following cell lines were maintained in RPMI-1640 or Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah) as previously described [4, 5]: human myeloid leukemia cell line HL60 [13], human B-cell-derived leukemia/lymphoma cell line 8392 [14], human melanoma cell line 242 [15], human lung cell adenocarcinoma line MV522 [16], and colon adenocarcinoma lines HT29 [17] and SW48 [18]. The human breast cancer carcinoma MDA231 and the adriamycin-resistant gp170/mdr1-positive daughter line MDA 3-1 were obtained from Dr. W. McGuire [19]. Human breast cell carcinoma lines MCF7/wt and doxorubicin-resistant anionic glutathione transferase-positive daughter line MCF7/ADR [20] were obtained from Dr. C.E. Meyers (MCF7/ADR displays an embryonic glutathione transferase that causes a 50-fold increase in cellular detoxification capacity of glutathione transferase). The human AML HL60 wild-type and doxorubicin-resistant gp180-positive cell line HL60/ADR were obtained from Dr. M. Center [21]. Resistance in this line is mediated by a 180 kDa glycoprotein called MRP that is distinct from the mdrl/gp170 protein. The KB line and daughter lines colchicine-resistant KB-Cl and vinblastine-resistant KB-V1 were obtained from Dr. M. Gottesman [22]. The murine leukemia line L1210 and the cisplatin-resistant daughter line L1210-DDPT, the BCNU-resistant line L1210-BCNU, the melphalan-resistant line L1210-PAM and the cyclophosphamide-resistant line L1210-CPA were obtained from Dr. D.P. Griswold Jr. [23]. The CEM T-cell line and daughter cell line VM-1 with altered topoisomerase II activity were obtained from Dr. W. Beck [24]. The 8266 myeloma line, melphalan-resistant daughter line 8226/LR5, and adriamycin-resistant daughter line 8226/DOX were obtained from Dr. W. Dalton [25]. The DC3F parent line and the DC3F/C10 daughter line with altered topoisomerase I were obtained from Dr. Y. Pommier [26]. The cytotoxic effects of illudin S were assessed as previously described using liquid culture assays, thymidine incorporation, and colony-forming assays (CFA) [4, 5].

Illudin S preparation

Omphalotus illudens subtype S.T. Carey 4435 (formerly *Clitocybe illudins*), was obtained from the New York Botanical Garden (New York, N.Y.) and illudin S prepared as previously described [1–3]. Radiolabeled illudin S was prepared by the addition to the fermentation broth of the precursor tritiated sodium acetate [5, 10]. The specific activity of radiolabeled illudin S used in this study was 245 mCi/mmol.

Illudin transport studies

Cellular uptake of tritiated illudin S was performed by incubating cells with radiolabeled illudin S in 1–5 ml total volume at 37 °C. After incubation cells were separated from the medium as previously described [27] by centrifugation through bovine serum albumin (BSA; Sigma Chemical Company, St. Louis, Mo.). The cell suspension was carefully layered onto this 10% BSA layer, centrifuged for 1 min (Beckman Microcentrifuge) and allowed to stop without braking. Cells were centrifuged into the lower layer while most of the radiolabeled illudin S remained in the upper layer. Aspiration at low pressure was used to remove first the upper and then the lower layer. Residual moisture was removed by touching the tip of a tissue wipe to the side of the pellet without disturbing the cells. Cells were lysed in AQUAMIX scintillation fluid (ICN Pharmaceuticals, Costa Mesa, Calif.). With this technique, background was less than 100 cpm. For thymidine uptake studies, the same procedure was used except cells were exposed to carbon-14-labeled thymidine. For measurement of efflux, after exposure to the tritiated illudine S, suspension cells were rapidly centrifuged, washed once, and resuspended in medium. For measurement of efflux with monolayer cells, the cells were rinsed rapidly three times with medium, then covered with medium. Aliquots of medium and cells were removed at specific times for counting in a liquid scientillation counter.

Analysis of data

Comparison of IC_{50} values between a parental cell line and single mdr daughter line was by Student's *t*-test. Comparison between a parental cell line and multiple mdr daughter lines was by ordinary ANOVA, followed by Tukey-Kramer multiple comparison post-ANOVA analysis. The analysis was performed using Instat Software version 2.02 (Graph Pad, La Jolla, Calif.). Comparison of IC_{50} values with cellular accumulation was by the Spearman Rank Correlation test, and the analysis was performed using Prism Software version 2.0 (Graph Pad).

Results

Cytotoxicity and cellular uptake studies

Seven commonly used tumor lines were chosen for detailed studies of intracellular illudin S uptake. All cell lines were markedly sensitive to illudin S using a 48-h exposure (Table 1). There was marked variation, however, in illudin S sensitivity between cell lines with a 2-h exposure. The IC_{50} values obtained from the CFA method (Table 1) correlated with the values obtained from thymidine incorporation. The intracellular accumulation of illudin S with a 2-h exposure at 100 ng/ml correlated with the 2-h IC_{50} values ($r = 0.97$, $P < 0.01$; (Fig. 1).

Table 1 Comparison of illudin S cellular uptake versus cytotoxicity in various human tumor cell lines

Cell line	2-h uptake[a] (picomoles)	2-h IC_{50}/CF[b] (n*M*)	2-h IC_{50}/Td[c] (n*M*)	48-h IC_{50}[d] (n*M*)
HL60	89 ± 2	8 ± 1	10 ± 1	3 ± 1
MV522	51 ± 5	79 ± 11	19 ± 6	4 ± 1
SW48	82 ± 6	21 ± 2	81 ± 4	8 ± 1
HT29	59 ± 6	32 ± 2	88 ± 4	6 ± 1
MDA231	55 ± 3	36 ± 3	2 ± 1	1 ± 0.1
MCF7	29 ± 4	115 ± 13	58 ± 5	10 ± 3
8392	14 ± 2	363 ± 21	236 ± 31	8 ± 2

[a] Picomoles per 10 million cells (mean ± SD of three experiments)
[b] Concentration producing a 50% decrease in colony formation with a 2-h exposure (mean ± SD of three experiments)
[c] Concentration producing a 50% decrease in thymidine incorporation into DNA with a 2-h exposure (mean ± SD of three experiments)
[d] Concentration producing a 50% decrease in cell count with a 48-h exposure (mean ± SD of three experiments)

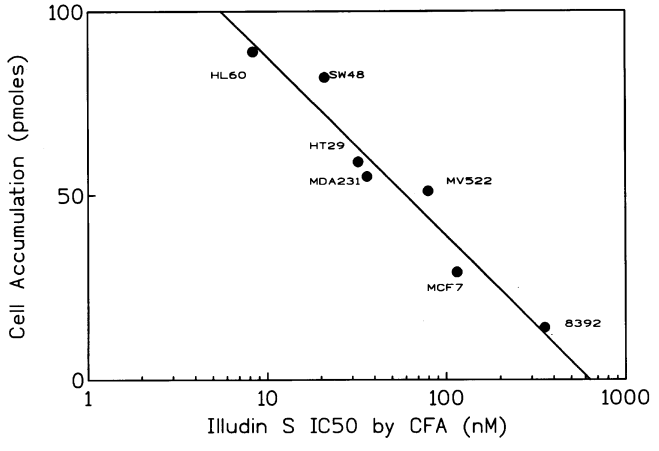

Fig. 1 Comparison of cellular accumulation of illudin S during 2-h exposures versus the IC_{50} values for 2-h exposures as determined using a colony forming assay. Data points are the mean of three experiments for both cellular accumulation and cytotoxicity studies

Table 2 Molecules of illudin S required for 50% inhibition of colony formation

Cell line	IC_{50}[a] (n*M*)	*Illudin S molecules*[b] (per cell)
HL60	8	78 000 ± 12 000
8392	363	852 000 ± 108 000
MDA231	36	516 000 ± 18 000
HT29	32	480 000 ± 18 000
SW48	21	452 000 ± 30 000
MCF7	115	1 140 000 ± 36 000

[a] Concentration producing a 50% decrease in colony formation with a 2-h exposure
[b] The number of molecules accumulated intracellularly as calculated by uptake or radiolabeled illudin S (mean ± SD of three to five determinations)

Tumor cells were incubated with illudin S at their respective IC_{50} values (derived from the CFA), and intracellular illudin accumulation determined. The number of intracellular illudin S molecules required to kill 50% of different tumor cells varied widely from 78 000 to 1 140 000 molecules per cell (Table 2). The number of illudin S molecules required to kill 50% of a tumor cell type correlated with the 2-h CFA IC_{50} values ($r = 0.943$, $P < 0.02$).

As the plasma half-life of illudin analogs in vivo is less than 15 min [28, 29], the toxicity of illudin S against the HL60 myeloid leukemia and MV522 lung carcinoma cell lines, which have been previously used for xenograft studies [11, 12], and the 8392 B cell leukemia/lymphoma cell line was studied using a 15-min exposure. Using this short exposure time, there was a marked difference in cell killing between the three cell lines. More than 99% of the HL60 and MV552 cells were killed with a 15-min exposure to 4 μ*M* illudin S (Fig. 2), but with 8392 B cells the IC_{50} value was not obtained even at a concentration of 150 μ*M* illudin S. Increasing concentrations of illudin S in the medium produced increases in uptake of illudin S in HL60 and MV522 cells and resulted in a marked decrease in cell survival (Table 3). There was no detectable illudin uptake by 8392 B cells over 15 min at 3.8 μ*M*. The number of molecules taken up by the MV522 cells with various exposure periods was determined. The number of molecules per MV522 cell producing a 50% inhibition (by CFA) appeared relatively constant over different time periods (Table 4).

Analysis of energy-dependent cellular accumulation

Cellular uptake studies were performed to determine the kinetic parametrs of illudin S energy-dependent uptake [5] in the three different cell lines. The HL60 and MV522 cells displayed energy-dependent cellular accumulation of illudin S that was saturable at high external illudin S concentrations (Fig. 3). Kinetic

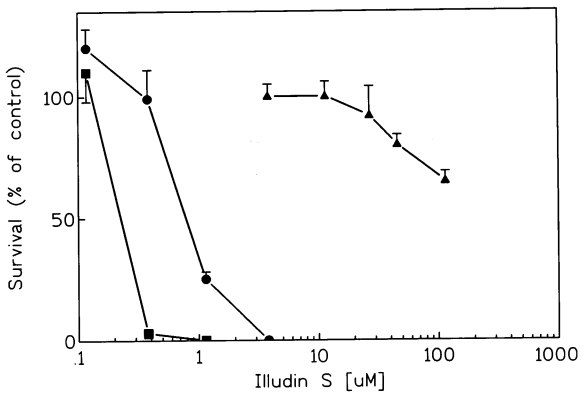

Fig. 2 Cytotoxicity of illudin S against human cell lines with a 15-min exposure as determined using a colony forming assay. Values are means ± SD of three experiments (■ HL60 myeloid leukemia cells, ● MV522 lung carcinoma, ▲ 8392 B cells)

analysis of uptake was performed using both the classical Michaelis-Menton linear method and a nonclassical nonlinear algorithm computer method [30]. Results for both methods were similar for all three cells lines. The V_m, K_m, and V_d values for the HL60 myeloid and MV522 carcinoma cells were relatively equivalent (Table 5). We previously have been unable to detect evidence of energy-dependent transport in 8392 B cells [5]. Using radiolabeled illudin S with a specific activity five times higher, and an incubation time four times longer than previously used, we detected evidence of a low capacity transport in 8392 B cells. The V_{max} for 8392 B cells was markedly below that of the HL60 and MV522 cell lines, and the K_m value was markedly higher. This suggests that at the low micromolar serum concentrations obtained for illudin analogs in vivo [28, 29], the energy-dependent transport of illudin S into 8392 B cells would be minimal.

Efflux and miscellaneous in vitro studies

Efflux studies were performed by exposing HL60 cells to radiolabeled illudin S at 100 ng/ml (28 n*M*) for 2 h, at which time uptake was 8.4 ± 1.0 pmol of illudin S per 10 million cells. Efflux was monitored at 15-min intervals, but release of radiolabeled illudin S was not detected even after 2 h. This suggests that the metabolic conversion of illudin S exceeded transport capacity at low concentrations. Therefore, HL60 cells were exposed to a high concentration of illudin S (46 μ*M*), in an attempt to overwhelm this metabolic conversion, and the studies repeated. No release of radioactivity was detected over 2 h. This concentration represents 200 times the IC_{50} value for a 15-min exposure, and 330 ± 30 pmol of illudin S uptake per 10 million cells.

As illudin S inhibits thymidine incorporation into DNA [4], and intracellular uptake occurs by an energy-dependent mechanism, it is possible that illudin competes with thymidine transport. Thymidine uptake

Table 4 Comparison of illudin S uptake and cytotoxicity with time in MV522 cells

Time (min)	IC_{50}[a]	Molecules per cell[b]
15	700 ± 120	1 640 000 ± 120 000
30	250 ± 50	1 840 000 ± 220 000
120	79 ± 5	1 720 000 ± 70 000

[a] Concentration producing a 50% inhibition in colony formation for the stated exposure period (mean ± SD of three determinations)
[b] Number of molecules accumulating in each cell at the given IC_{50} value for that exposure period (mean ± SD of three determinations)

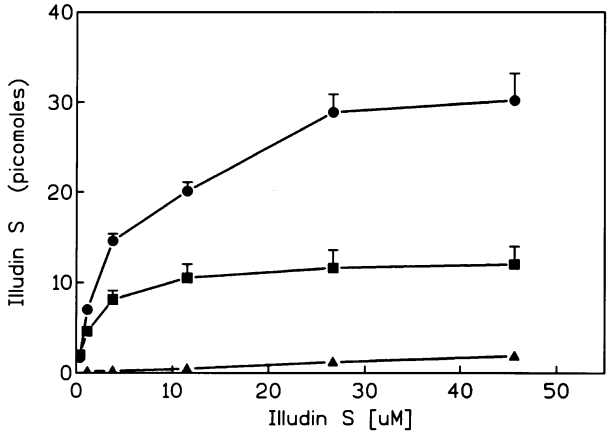

Fig. 3 Energy-dependent cellular accumulation of illudin S as a function of external illudin S concentrations with a 15-min exposure, derived as the differences between uptake at 37 °C and at 4 °C. Values are means ± SD of three experiments (■ HL60 myeloid leukemia cells, ● MV522 lung carcinoma, ▲ 8392 B cells)

Table 3 Comparison of illudin S uptake and cytotoxicity versus concentratioin with 15-min exposure (*ND* not detectable)

ng/ml	n*M*	Colony formation (% of control)			Uptake (pmol/10 million cells)		
		HL60	MV522	8392	HL60	MV522	8392
100	382	67	74	100	29	27	ND
300	1146	0.6	31	104	65	110	ND
1000	3820	0.05	0.2	98	160	234	ND

Table 5 Kinetic analysis of illudin S energy-dependent cellular accumulation (V_m in pmol/min for 10 million cells, K_m in μM, V_d in min^{-1} for 10 million cells when S expressed in μM). Nonlinear regression analysis was performed using the KINETICS software [30]

Cell line	Parameter	Michaelis-Menton linear analysis	Nonlinear algorithm
HL60	V_m	27.8 ± 2.0	29.0 ± 5.1
	K_m	7.1 ± 1.5	7.1 ± 1.6
	V_d	0.34	
MV522	V_m	33.6 ± 1.6	34.8 ± 3.0
	K_m	5.6 ± 0.9	6.0 ± 1.4
	V_d	0.13	
8392	V_m	6.0 ± 2.6	3.0 ± 0.9
	K_m	81.0 ± 5.0	25 ± 2
	V_d	0.05	

into cells in the presence of illudin S was analysed, but illudin S did not inhibit thymidine uptake. HL60 and 8392 cells were preincubated at 37 °C for 30 min in the presence or absence of 100 ng/ml illudin S which is sufficient to inhibit thymidine incorporation into DNA by more than 85% in HL60 cells. HL60 and 8392 cells inhibited without illudin S displayed thymidine uptake of 2.12 ± 0.10 and 2.35 ± 0.12 pmol per million cells in 10 min (mean ± SD, $n = 3$), respectively. HL60 and 8392 cells incubated with illudin S displayed thymidine uptake of 2.35 ± 0.12 and 1.97 ± 0.14 pmol per million cells in 10 min (mean ± SD, $n = 3$), respectively.

MDR cell line studies

It has been previously noted that CEM mdr cell lines remain relatively sensitive to illudin [4] despite marked resistance to conventional anticancer agents. The mechanisms of resistance for these mdr daughter lines have now been elucidated. All mdr cell lines tested were sensitive to illudin S, regardless of whether resistance was due to gp170 expression, gp180/MRP expression, GSHTR-pi expression, alternations in topoisomerase I or topoisomerase II activity, or increased ability to repair DNA damage (Table 6). It was noted that one resistant cell line (8226/LR5) was more sensitive to illudin S than the parent 8226 cells ($P < 0.001$). The MCF7/ADR cell line, resistant by virtue of expression of GSHTR-pi [20], was partially resistant to illudin S, although this resistance did not reach statistical significance.

The relationship between illudin S uptake and toxicity was further evaluated in the MCF7, MCF7/ADR, 8226, 8226/LR5, and 8226/DOX cell lines using the CFA method for toxicity (Table 7). MCF7/ADR cells were not less sensitive than MCF7 parent cells using the more stringent CFA method of cytotoxicity assessment. There was also no difference in cellular uptake of illudin S between MCF7 and MCF7/ADR cells. The

Table 6 Illudin S toxicity against various mdr lines

Cell line	Resistance mechanism	48-h IC_{50}[a] (nM/l)
CEM (T-cell) parent		11 ± 1
VM-1	Topoisomerase II	13 ± 1
MDA-231 (breast) parent		0.9 ± 0.2
3-1	gp170/mdr1	0.9 ± 0.4
MCF7 (breast) parent		0.9 ± 0.1
ADR	GSHTR-pi	3 ± 1
HL60 (myeloid) parent		3 ± 1
ADR	gp180/MRP	2 ± 1
KB (epidermoid) parent		0.6 ± 0.1
C-1	gp170/mdr1	0.7 ± 0.2
VBL	gp170/mdr1	0.7 ± 0.2
2008 (ovarian) parent		55 ± 5
Cisplat	DNA repair	52 ± 3
L1210 (murine) parent		0.4 ± 0.1
DDPT (cisplatin)		0.5 ± 0.1
BCNU		0.6 ± 0.1
PAM (melphalan)		0.5 ± 0.1
CPA (cycloplas)		0.5 ± 0.1
8226 (myeloma) parent		316 ± 53
LR5	Thiol content	6 ± 1 ($P < 0.001$)
DOX		218 ± 20
DC3F (murine) parent		24 ± 7
C-10	Topoisomerase I	17 ± 3

[a] Concentration producing a 50% decrease in cell count with a 48-h exposure (mean ± SD for three to five determinations)

Table 7 Comparison of illudin S toxicity and uptake

Cell line	CFA IC_{50}[a] (nM)	Cellular uptake[b] (pmol/10 million cells)
8226	13 100 ± 3200	13 ± 1
8226/DOX	12 900 ± 2700	13 ± 1
8226/LR5	164 ± 19	46 ± 6
MCF7	115 ± 13	29 ± 4
MCF7/ADR	188 ± 26	31 ± 4

[a] Concentration producing a 50% inhibition in colony formation for a 2-h exposure to illudin S (mean ± SD for three determinations)
[b] Picomoles of illudin S accumulating in 2 h in 10 million cells exposed to the IC_{50} concentration (mean ± SD for three determinations)

8226/LR5 daughter line demonstrated increased illudin S uptake when compared with parental 8226 cells (Table 7), in agreement with the increased sensitivity of the 8226/LR5 cells to illudin S (Tables 6, 7).

Discussion

Illudins are cytotoxic to a variety of human tumor cells at nano- to low micromolar concentrations using only

a 2-h exposure. In contrast, killing of other types of tumor cells, normal fibroblasts and normal bone marrow progenitor cells requires exposure to high micromolar concentrations of the toxins for 2 h or more [4]. We have now demonstrated that sensitive tumor cells are killed by 15-min exposures to micromolar concentrations (Fig. 2). There was a 15-fold variation in the number of illudin S molecules required to kill 50% of the cells from a specific tumor line (Table 2), and the number of molecules was correlated with the 2-h CFA IC_{50} ($P < 0.02$; Table 1, Fig. 1). Interestingly, the number of molecules of illudin S per MV522 cell producing a 50% inhibition (by CFA) was constant over different times (Table 4). This suggests that the cellular process responsible for repairing illudin-induced damage is overwhelmed at a specific point. The concept that the cellular process for repair of illudin-induced damage can be overwhelmed is further supported by comparing increased uptake versus increased toxicity. The 8226/LR5 daughter line demonstrated only a mild increase in illudin S uptake, but was markedly sensitive as compared to the parental 8226 line (Table 7) and HL60 cells (Table 3).

At low concentrations of illudin S, illudin uptake was correlated with cytotoxicity. We have demonstrated that uptake is saturable, temperature-sensitive, and energy-dependent [5] with low K_m and relatively high V_m values in sensitive cells such as HL60 and MV522 (Fig. 3, Table 5). In contrast, the relatively resistant 8392 B cell leukamia/lymphoma cells displayed a fivefold lower V_m and a tenfold higher K_m value. At the low micromolar concentrations, such as detected after intravenous administration of acylfulvene in mice or dogs [28, 29], relative uptake in sensitive tumor cells would be expected to be high whereas uptake in resistant tumor cells would be relatively low. Although we did not examine cellular uptake into either mortal human diploid or immortalized (transformed) cells such as fibroblasts, we have previously noted that such cells are resistant to illudin S to an extent [4] similar to the 8392 B cells examined here. Thus, after intravenous administrations into a tumor-bearing host, one would expect high drug uptake in sensitive tumor cells, but minimal accumulation into normal cells, resulting in selective cytotoxicity of the drug against the tumor cells.

We did not detect evidence of efflux of radiolabeled drug (either as unchanged illudin S or as metabolite) from HL60 cells even using concentrations 200 times the IC_{50} value. This lack of detectable efflux of illudin S could be a result of intracellular metabolism, in agreement with previous reports by others [31, 32], but could also occur as a result of binding of illudin S to intracellular components.

A major potential clinical advantage of illudin-derived compounds is that a variety of the mdr cell lines, regardless of mechanism of resistance, remain sensitive to the agents (Table 6). The present studies expand and confirm prior studies showing activity of illudins against $gp170^+$ drug-resistant cells derived from one tumor type [4]. The present studies also demonstrated that in vitro sensitivity to illudins was correlated closely with drug uptake, and that there was no detectable efflux of radiolabeled analog from cancer cells. Illudins fail to directly bind to purified DNA in vitro, but will bind covalently to intracellular DNA M.J. Kelner and T.C. McMorris, unpublished results. The latter findings suggest that the compounds are converted intracellular to a reactive intermediate that covalently binds to DNA, in agreement with previous reports of microsomally mediated metabolism of illudin S to a reactive species [31, 32]. The mechanism of illudin uptake is of interest in understanding how tumor cells may process natural products which are not substrates for gp170 or other known transport proteins.

The information obtained in this study could be used to design clinical phase I trials, and to develop new analogs with improved therapeutic indexes. The short exposure times noted here to kill tumor cells suggests that the drug could be administered rapidly, so that the need to achieve prolonged exposure via infusions could be avoided. Development of new analogs with improved therapeutic indexes could focus on increasing the difference in cellular accumulation (V_i) between target or tumor cells (e.g. MV522) and nontarget cells (e.g. 8392). This could be achieved by designing analogs with larger variations in V_{max} or K_m parameters between target and nontarget cells.

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