Acta Oncologica Vol. 34, No. 2, pp. 157–164, 1995

IN VIVO EFFICACY OF NOVEL SYNTHETIC ENEDIYNES 1

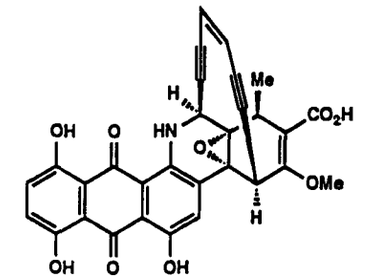
WOLFGANG WRASIDLO, ANDREWS HIATT, STEVEN MAUCH, ROBERT A. MERLOCK and K.C. NICOLAOU

We have investigated the biodistribution, toxicity, and antitumor activity of a new type of synthetic compound containing an enediyne functional group capable of benzenoid diradical generation. The design of this cytotoxic molecule was based on the structures of naturally occurring enediyne antibiotics. Compared to the natural compounds, the synthetic enediyne displayed cytotoxicities approaching the natural analogs. Using a tritiated analog, biodistribution studies revealed relatively high uptake levels in kidney, lung, heart, and spleen with moderate levels in all other organs. Antitumor activity was apparent, with significant tumor regression observed in athymic nude mice with established M21 melanomas. Significant tumor antiproliferative effects were observed against L-1210 mouse leukemia, A549 lung carcinomas and PC3 prostate carcinomas in athymic nude mice, and against EMT-6 mouse mammary adenocarcinomas in Balb/cByJ mice. These results suggest that synthetic enediynes may be useful therapeutic compounds since their design reduces systemic toxicity compared to the natural products, without compromising antitumor activity. The relatively low sensitivity of many established cell lines to synthetic enediynes suggests a discrepancy between cell culture and in vivo tumor cytotoxicities. Adaptation of some cell lines for in vivo proliferation may affect their sensitivity to synthetic enediynes.

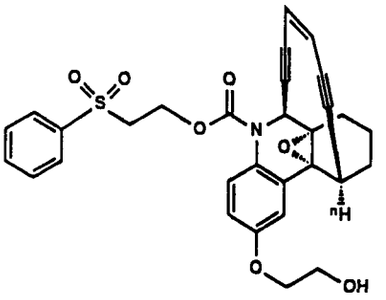
The naturally occurring enediyne antibiotics consist of calicheamicin (1, 2), esperamicin (3, 4), neocarzinostatin (5–8), dynemicin (9) and more recently kedarcidin (10). Despite the significant structural diversity represented by these compounds, they are thought to be cytotoxic by a common mechanism involving rearrangement of the enediyne to produce an arenyl or indenyl diradical (11, 12). In vitro DNA cleavage experiments have further suggested that the mechanism of induced cell death involves abstraction of hydrogen atoms from cellular DNA resulting in scission of the phosphodiester backbone (13–16). The antitumor activity of these compounds (17, 18) has inspired synthetic strategies (19) whereby analogs and hybrids containing the enediyne core structure are coupled with various other structural features serving to enhance their biological activity. The synthetic analogs have been used to further investigate both the morphology of cell death as well as the structural determinants which are necessary for biological activity (20, 21). In these studies, various tumor cell lines were tested for their sensitivity to the synthetic compounds. Whereas dynemicin was generally cytotoxic to all of the cell lines tested, some of the synthetic enediynes were found to be selectively toxic especially to leukemic cells. Enediyne 1 (Fig. 1), for example, had an IC_{50} of $\sim 10^{-14}$ M against the Molt-4 T cell leukemia line. The morphology and DNA degradation of cells exposed to enediyne 1 clearly indicated apoptosis as the mechanism of cell death (21). The absence of a minor groove binder or intercalative capability of this enediyne suggested that a direct attack on the DNA phosphodiester backbone was not a primary factor in its mechanism of cytotoxicity. Indeed enediyne 1 is only capable of very limited DNA cleavage in vitro given prolonged incubation

Received 11 February 1994.
Accepted 20 September 1994.
From the Department of Cell Biology (A. Hiatt), Department of Chemistry (K.C. Nicolaou), and Department of Molecular and Experimental Medicine (S. Mauch, R.A. Merlock, W. Wrasidlo), The Scripps Research Institute, La Jolla, and Department of Chemistry (K.C. Nicolaou), University of California, San Diego, La Jolla, California, USA.
Corresondence to: Dr. W. Wrasidlo, Dept. of Molecular Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.
Paper presented at 18th International Congress of Chemotherapy, Stockholm, Sweden, June 27–July 2, 1993.

 ISSN 0284-186X

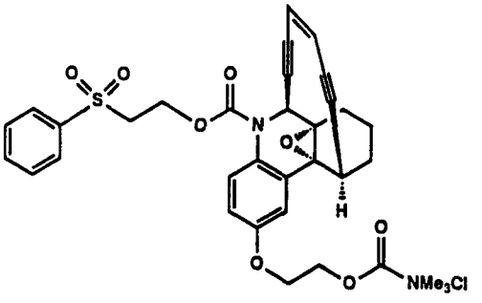


Dynemicin A



Synthetic enediyne 1: n=1

Synthetic enediyne 2: n=3



Synthetic enediyne 3

Fig. 1. Chemical structures of dynemicin and two synthetic enediynes.

times (20). The possibility that the cellular target of enediyne 1 was something other than cellular DNA was further suggested by the enantiomer dependence of cytotoxicity and by the unusual biological activity of analogs which are incapacitated from undergoing aromatization and diradical formation. Exposure of Molt-4 cells to these non-reactive analogs prevents these cells from undergoing apoptosis. This suggests that the target of these enediynes plays a role in regulating the pathway of cell death (21).

Due to the apparent selective nature involved in the cytotoxicity of enediyne 1 against established cell lines, it is of great interest to understand the extent to which this level of selectivity is maintained among tumor cells in vivo. To this end, we have investigated the biodistribution, toxicity, histology, and antitumor activity of enediyne 1. The antitumor activity of an analog, compound 3, has also been investigated in mouse models expressing human tumors. Significant antiproliferative responses to the enediynes were observed in mice expressing solid tumors, including A549 lung adenocarcinomas and PC3 prostate carcinomas in athymic nude mice, and EMT6 mammary adenocarcinomas in Balb/cByJ mice. Evaluation of enediyne antitumor activity against M21 melanomas using single injection protocols revealed significant regression of established tumors in the athymic nude mice.

The significance of these results with respect to the relative expression of sensitivity to endiynes by established cell lines or tumor cells in vivo is discussed. In general, we have found that resistance to enediyne cytotoxicity in established cell lines may be abrogated upon passage of those cells as tumors in vivo.

Material and Methods

Chemicals. The enediynes were synthesized as previously described (22). Cell culture grade dimethylsulfoxide from Sigma Chemicals was used as a solvent for enediynes. Cell culture nutrients and reagents (fetal bovine serum, RPMI Kaighns Nutrient and Dulbeccos phosphate buffered saline solutions) were purchased from Irvine Scientific, Santa Ana, Ca. All tissue culture solutions were filtered through 0.2 micron membrane filters prior to use.

Cell lines. HT-29, A-549, and PC3 cell lines were obtained from American Type Culture Collection; the M-21 cell line was received from Dr. Ralph Reisfeld at The Scripps Research Institute, La Jolla, California, EMT-6 mouse mammary adenocarcinomas were propagated in vivo and were obtained from the Department of Radiology, University of California San Diego.

Tumor systems. Experimental tumor models were generated from cell lines which were maintained in a tissue culture facility under the standard cell proliferation conditons (37°C, 5% CO_2 in air). In the case of the EMT-6 tumors, cells were propagated in mice. The procedures used for the maintainance of tumors and the experimental details were according to protocols set down by the Developmental Therapeutics Programme, National Cancer Institute. The strain of mice, inoculation size, and site and administration route are given in the results and figures.

Chemotherapy. Hemocytometer counted cells suspended in Hanks medium (Gibco, Grand Island, N.Y.) were implanted intraperitoneally (i.p.) (0.5 ml/mouse). The test compounds were injected i.p. Solid tumor growth was measured periodically in two dimensions with slide calipers. Tumor volume was determined by the equation length × width^2 × (3.14159/2) and expressed in mm^3.

Biodistribution of radiolabelled enediyne. Tritiated enediyne was synthesized as described for the non-radioactive analog (22). Introduction of the tritium label was successfully achieved using a newly developed reagent, [^{3}H]nBu_3SnH, obtained from the National Tritium Labelling Facilities, Lawrence Berkeley Laboratories. The specific activity of a 10:1 molar ratio of a mixture of carrier 1 to radiolabelled enediyne 2 was 3 Ci/mmole. Balb/C mice, 6 weeks old, were injected i.p. with [^{3}H]enediyne 2 at 5 mg/kg of animal weight in 30% aqueous dimethylsulfoxide which was buffered to a pH of 7.3 with sodium phosphate. The tritiated compound was present at a dose of 4×10^7 cpm/animal. Mice were sacrificed in groups of 4 per experimental time point.

Results

Comparison of the cytotoxicities of naturally occurring and synthetic enediynes in mice

IC_{50} values against a panel of cell lines have been performed to estimate the extent of cell specific cytotoxicity (20). In these assays, the IC_{50} (M) for calicheamicin ranged from 10^{-5} M (M-21 melanoma line) to 10^{-12} M (Molt-4 leukemia line). Similarly, enediynes 1 and 3 had a range of 10^{-6} M (M-21 melanoma line) to 10^{-14} M (Molt-4 leukemia line). Dynemicin cytotoxicity showed little cell type specificity with IC_{50} values generally in the range of 10^{-8} to 10^{-9} M. These results suggest that the selective toxicity of the synthetic enediynes could have resulted in part from removal of the intercalating anthraquinone moiety of dynemicin or from the alternative mechanism involved in triggering cycloaromatization. The cell selective toxicity of the synthetic enediynes was qualitatively similar however to calicheamicin.

Biodistribution of enediyne 1 in normal mice

A derivative of enediyne 1 was synthesized in which the hydrogen at the enediyne brigehead was substituted with tritium. The specific activity of this compound was approximately 3 Ci/mmol. Positioning of the tritium atom in the backbone of the molecule ensured that the radiolabel would not be eliminated upon aromatization of the enediyne group.

Normal Balb/C mice were used to investigate biodistribution by injection of 4×10^7 cpm of tritiated enediyne at a dose of 5 mg/kg. Animals were sacrificed in groups of four after 15 min, 1, 3, 7, 24, and 48 h, the organs were surgically removed, rinsed with saline, minced, and dissolved in hyamin hydroxide. Radiolabel in each organ was determined by scintillation counting (Table). The blood level of enediyne showed the highest concentration at 15 min (2.4×10^6 cpm/ml) followed by an exponential decline to a steady state level (2×10^5 cpm/ml) by 48 h. The highest levels of enediyne uptake were observed in kidneys, lung, heart and spleen, with moderate levels of uptake in the other organs.

Antitumor activity of enediynes in mouse models

EMT-6 tumors in Balb/cByJ mice. Subcutaneous EMT-6 mouse mammary adenocarcinoma allografts were initiated using EMT-6 cells derived from carrier mice. The inoculum consisted of 1×10^5 cells in 0.2 ml RPMI. Animals were treated with 10 mg/kg enediyne 1 on days 1, 5, and 9. Tumor volume was measured on days 8, 10, 14, 16, 18, and 20 using an average of six mice per time point (Fig. 2a). The mean tumor volume of saline treated controls at day 20 was 1316 mm^3 compared to the enediyne-treated animals where the mean volume was 198 mm^3. At all time points the mean tumor volume of the treated animals was significantly less than the control ($p < 0.01$ on day 8 and $p < 0.001$ subsequently). Consequently, the therapeutic index (mean tumor $volume_{saline}$/mean tumor $volume_{enediyne}$) increased progressively and ranged between 3.9 and 6.6 from days 8 to 20.

A549 lung adenocarcinomas in athymic nude mice. Animals were injected in each axcillary flank with

Table

Biodistribution of enediyne 2 in Balb/C mice

Organ	Hours after injection (μg enediyne 2 per gram tissue[a])					
	0.25	1	3	7	24	48
Brain	0.28 ± 0.03[b]	0.11 ± 0.01	0.20 ± 0.03	0.18 ± 0.02	0.23 ± 0.02	0.25 ± 0.03
Heart	10.3 ± 1.40	0.54 ± 0.07	1.30 ± 0.20	0.37 ± 0.04	0.50 ± 0.07	2.10 ± 0.15
Kidney	10.6 ± 1.50	0.52 ± 0.60	0.62 ± 0.08	0.22 ± 0.03	0.13 ± 0.02	0.53 ± 0.10
Liver	2.57 ± 0.45	3.50 ± 0.45	3.75 ± 0.50	1.96 ± 0.35	1.77 ± 0.30	1.57 ± 0.20
Lung	8.47 ± 1.40	0.19 ± 0.03	0.50 ± 0.10	0.32 ± 0.08	0.06 ± 0.03	0.18 ± 0.04
Spleen	5.31 ± 1.05	0.66 ± 0.15	0.66 ± 0.10	0.33 ± 0.05	0.22 ± 0.04	0.39 ± 0.05
Thymus	2.86 ± 0.55	0.79 ± 0.15	2.07 ± 0.50	0.71 ± 0.15	0.74 ± 0.15	1.43 ± 0.35

[a] The amount of [3H]enediyne in each organ was calculated from scintillation counting of dissolved tissue as described in 'Material and Methods'. Four animals per experimental time point were used. [b] Values are expressed as the mean ± S.E.

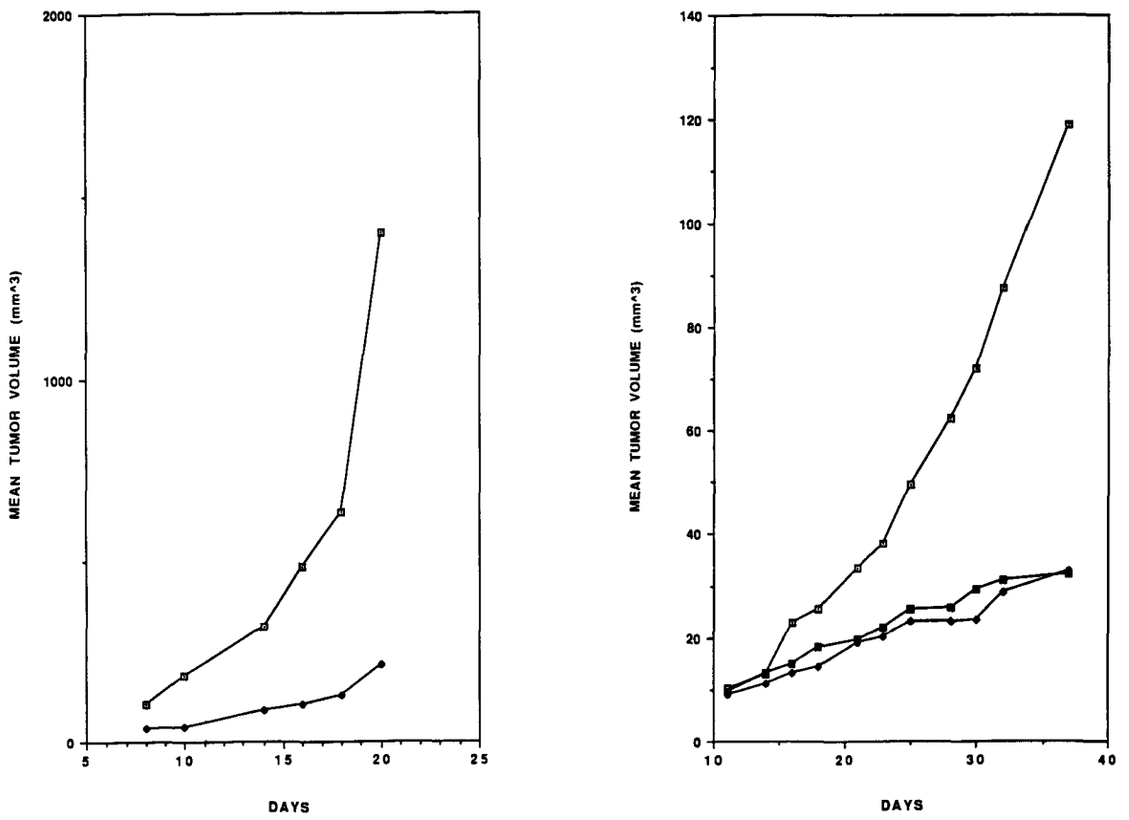


Fig. 2. a) Effect of enediyne 1 on EMT6 tumor growth. Subcutaneous EMT-6 mouse mammary adenocarcinoma allografts were initiated using EMt-6 cells derived from carrier mice. The inoculum consisted of 1×10^5 cells in 0.2 ml RPMI. Animals were treated with 10 mg/kg enediyne 1 on days 1, 5, and 9. b) Effect of enediynes 1 and 3 on A549 lung adenocarcinomas. Animals were injected in each flank with 4.25×10^5 cells in 0.13 ml RPMI on day 0. Treatments with 7.5 mg/kg enediyne 1 or 2 in DMSO occurred on days 1, 3, 5, and 7. In each graph, control animals are indicated by the open squares □, enediyne 1 by closed diamonds, ◆, and enediyne 3 (graph B) by closed squares, ■. Mean tumor volume was recorded in mm^3.

4.25×10^5 cells in 0.13 ml RPMI on day 0. Treatments with 7.5 mg/kg enediyne 1 or 2 in DMSO occurred on days 1, 3, 5, and 7. Tumor volumes were measured on alternate days up to 38 days using 6 animals per time point (2 tumors per mouse; $n = 12$) and were compared to control animals injected with saline alone. A significant antiproliferative effect was observed for both enediyne 1 and 3 as a result of the treatment at each point (Fig. 2b). The control to treatment ratio increased progressively from the start of enediyne treatment reaching a value of ~3.6 for both 1 and 3. Similar experiments were performed to determine the therapeutic window for enediyne treatment against the lung carcinomas. At 3 mg/kg, enediyne treatment was completely ineffective; significant therapeutic effects were observed at 5, 7.5 and 10 mg/kg. At 5 mg/kg, it appeared that enediyne 3 was more effective than 1 since the therapeutic indices were 3.11 and 1.73 respectively. At the higher levels of enediyne a therapeutic index of ~3.6 was observed by day 36 for both compounds.

PC-3 prostate carcinomas in athymic nude mice. Tumors were initiated by injection of 2×10^5 tissue culture grown PC3 cells; treatments were on day 1 after tumor initiation and were repeated on days 3, 7, 9, and 11. In these experiments, a comparison was made among the two synthetic enediynes and suramin, a drug used for treating refractory prostate carcinoma (23). The enediynes were administered at a dose of 5 mg/kg and suramin at 60 mg/kg. Mean tumor volume was measured on days 3, 8, 11, 14, and 16 (Fig. 3a). A general antiproliferative effect was observed with both enediynes but not with suramin. Between days 3 and 8, treatment with both enediynes resulted in a modest tumor regression of 27% and 38% for 1 and 3 respectively which was statistically significant ($p < 0.05$) in the case of 3. The therapeutic indices were 2.5 and 4.3 on day 16 for 1 and 3 respectively.

Established M21 melanomas in athymic nude mice. Mice were injected with tissue culture grown M21 cells on day 0. On day 14, tumor establishment was apparent and treatment was initiated with a single injection of enediyne 1 at 15 mg/kg or control. The mean tumor volume was determined each week using an average of 4 mice per measurement. Significant tumor regression was observed in the

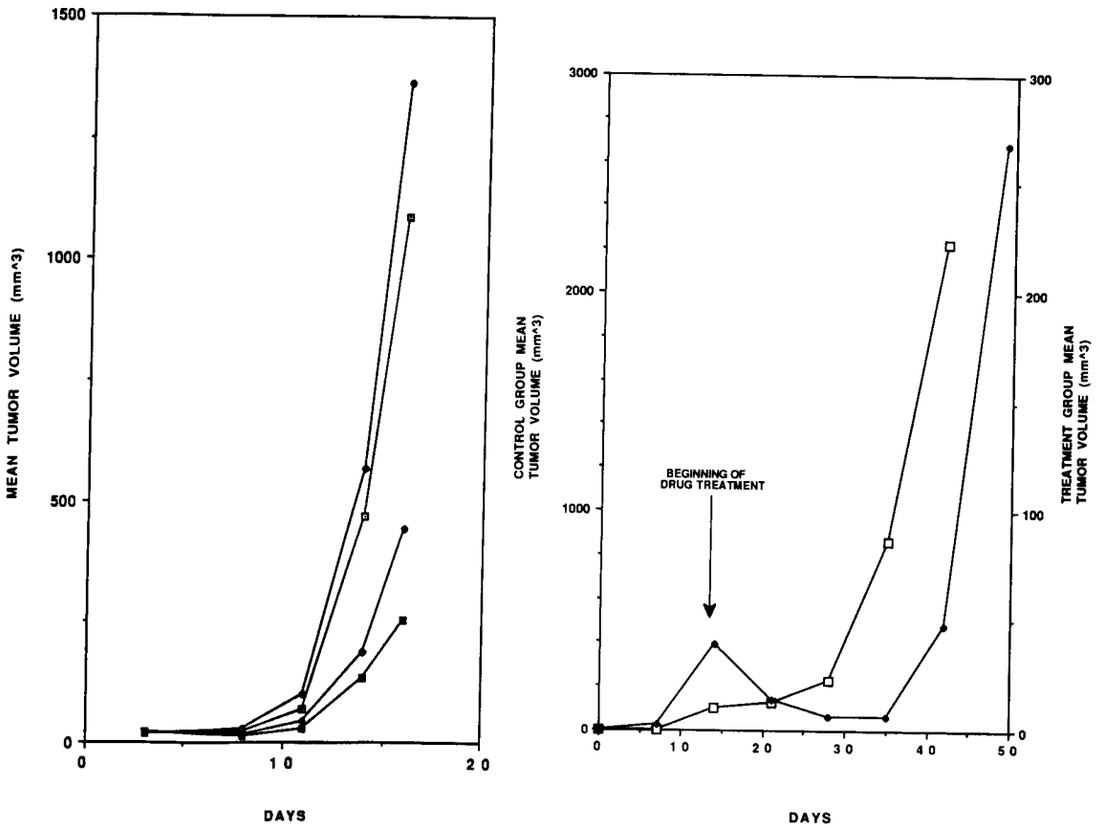


Fig. 3. a) Effect of enediynes 1 and 3 on PC3 prostate carcinomas. Tumors were initiated by injection of 2×10^5 tissue culture grown PC3 cells; treatments were on day 1 after tumor initiation and were repeated on days 3, 7, 9 and 11. The enediynes were administered at a dose of 5 mg/kg and suramin at 60 mg/kg. b) Effect of enediyne 1 on established M21 melanomas. Mice wer injected with 2×10^5 tissue culture grown M21 cells on day 0. On day 14, tumor establishment was apparent and treatment was initiated with a single injection of enediyne 1 at 15 mg/kg or control. In each graph, control animals are indicated by the open squares, □, enediyne 1 by closed diamonds, ◆, enediyne 3 (graph A) by closed squares, ■, and suramin (graph A) by open diamonds, ◇. Mean tumor volume was recorded in mm^3.

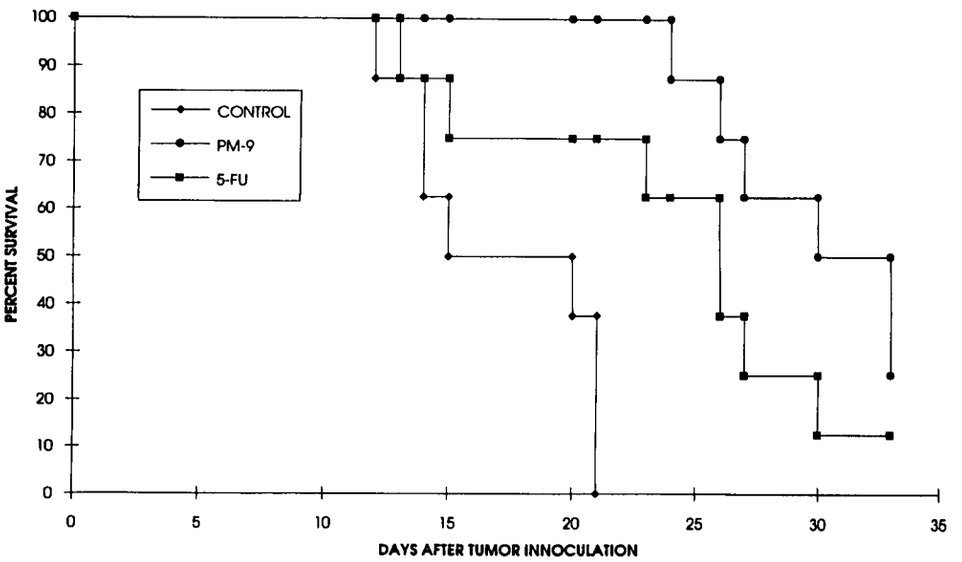


Fig. 4. Effect of enediyne 1 and 5 FU on L-1210 mouse leukemia. Tumors were initiated by i.p. injection of 1×10^6 tissue culture grown L-1210 cells and treatments were on day 1, 3, 5, and 7 for enediyne 1 at a dose of 7.5 mg/kg and on day 1 for 5-FU on day 1 at a dose of 200 mg/kg.

experimental animals during weeks 3–5, representing an 84% reduction in tumor volume at week 5 at which time the therapeutic index reached a peak of 148. Mean volume of tumors at week 6 was approximately 2% of the control values (Fig. 3b).

HT-29 colon carcinomas in athymic nude mice. In contrast to the results obtained above, we found no significant antiproliferative effect of enediyne 1 on this tumor model. Animals were inoculated with 1×10^5 cells in 0.5 ml RPMI on day 0 and were given 7 mg/kg enediyne 1 on days 1, 4, and 9. By day 16, all test animals displayed obvious signs of poor health and no significant antitumor activity was detected.

L-1210 leukemic ascites survival in CD2F1 mice. Mice in groups of 8 were injected i.p. with $1 \times$ E6 tissue culture grown L-1210 cells in 0.1 ml RPMI media. Mice were treated with enediyne 1 on day 1, 3, 5, 7 at a dose of 7.5 mg/kg and with a single dose of 5 FU of 200 mg/kg on day 1, which is the standard positive control for this cell line. As can be seen from the survival data in Fig. 4, both compounds showed antiproliferative effects over the saline control with T/C values for the 5-FU of 166% and for the enediyne of 233%.

Histological evaluation of animals receiving therapeutic doses of enediyne 1. Athymic nude mice were injected subcutaneously with A549 cultured lung tumor cells and were treated with enediyne 1 or saline as described above. The appearance of the tissues among the control and experimental animals was generally the same except for the appearance of the spleens in the experimental animals which appeared to be undergoing moderate follicular reaction.

Discussion

The purpose of this study was to evaluate the antitumor activity of a new class of synthetic compound derived from naturally occurring antibiotics. These synthetic enediynes have previously been evaluated for their cytotoxicity against a panel of human and mouse tumor cell lines (20). From these results, it was predicted that enediyne 1 would be primarily effective against leukemias in vivo since the IC_{50}s against human T cell, B cell, and mouse leukemia cell lines ranged from 1.1×10^{-9} to 2.0×10^{-14} M. Indeed the results with a mouse leukemia cell line, which gave a IC_{50} value of 1.1×10^{-9} (Fig. 4) showed enediyne 1 to exhibit superior antitumor behavior compared with 5-FU which is the standard for comparison with this leukemia model. In contrast, the IC_{50}s against either melanoma or colon carcinoma cell lines was in the range of $1.6–3.1 \times 10^{-6}$ M. The greater than thousand-fold lower cytotoxicity against the melanoma and colon carcinoma cell lines suggested that enediyne 1 would not be as therapeutically effective against these tumors in vivo compared to leukemia. This prediction was borne out in the case of the HT-29 colon carcinomas but the regression of established melanomas given as a single dose of enediyne 1 could not have been predicted from the in vitro test results. The relative toxic effects observed the HT-29 study was surprising, since in all other solid tumor model experiments the enediynes were very well tolerated with greater than 90% survival at the end of the studies.

Whereas no established mammary tumor cell lines have been tested against enediyne 1, the human breast carcinoma cell line MCF-7 was of intermediate sensitivity with an IC_{50} of $\sim 1 \times 10^{-6}$. Significant antiproliferative effects of enediyne 1 against EMT-6 mouse mammary adenocarcinomas were observed. Similarly, the IC_{50} of enediyne 1 against a variety of lung carcinoma cell lines displayed intermediate toxicity, being in the range of 10^{-7} M. However, significant antiproliferative effects against A549 lung adenocarcinoma xenografts were also observed for at least two weeks after treatments had ceased.

Due to the limited availability of 3 this enediyne could not be included in all of the experimental protocols. Our preliminary results with enediyne 3, however, suggest that modification of the glycol tether by the addition of the charged trimethyl amine may enhance the efficacy of the enediyne due perhaps to an increase in tumor uptake. The trimethyl amine moiety does not measurably affect the reactivity of the enediyne in terms of its potential for cycloaromatization, but it greatly increases its solubility in aqueous media.

In general, these results suggest that the selective cytotoxicity of synthetic enediynes against established tumor cell lines is not predictive of their apparently broader efficacy against the corresponding tumors in vivo.

One explanation to account for the apparently high sensitivity of the melanomas in vivo is a relative increase in accessibility of the drug to the tumors in this particular model. Future experiments using multiple dose schedules must address the crucial issue of optimizing the access of endiynes to tumors. However, if accessibility is a limiting factor in the A549, EMT6 or PC3 models, our results are underestimating the sensitivity of these tumors to enediyne therapy. In addition, since we have utilized a variety of protocols for administering the enediynes, our results indicate that enediyne therapy after establishment of solid tumors may be more effective than multiple injections during tumor establishment. Future experiments will have to address this possibility with a variety of solid tumor models.

Allthough our results are very preliminary at this time they do suggest that the M-21 melanoma tumors constitute an ideal model for evaluating the physiology of tumor regression induced by enediynes and for optimizing the primary factors responsible for induction of tumor regression. In a variety of model systems, it has been observed that regression of established tumors can occur by a

mechanism involving apoptotic cell death (24–27). Although the metabolic pathways controlling this process have yet to be characterized, apoptosis may be a useful morphological marker for understanding the control of cell death both in vitro and in vivo. Enediyne-induced cell death in culture clearly occurs by a mechanism of apoptosis (21). This is especially apparent in those cell lines which are most sensitive to enediyne 1, such as the Molt-4 and HL-60 leukemia lines. In cell lines which are relatively insensitive to endiyne 1, such as the melanomas, apoptosis is not apparent. Consequently, it will be of considerable interest to identify the morphological characteristics of established melanomas during regression induced by the enediyne treatment. This sytem will be useful in determining whether a propensity for apoptotic cell death can be determined by the aggregate state of tumor cells.

Our previous results have demonstrated that naturally occurring enediynes, such as calicheamicin and dynemicin, can also efficiently induce a program of apoptotic cell death (21). Both calicheamicin and dynemicin induce this morphology in Molt-4 cells at the same concentrations as enediyne 1. The natural enediyne antibiotics are known to have potent antitumor properties (9, 18). Calicheamicin, for example, yielded T/C values in excess of 175 against P3888 leukemia or B-16 melanoma models at concentrations between 1.25 and 2.5 μg/kg (18). Unfortunately, none of the native calicheamicins have been developed for clinical use largely because of unacceptible, delayed organ toxicity in animals (G.A. Ellestad, personal communication). Our results indicate that structural modifications resulting in an alternative triggering of chemical reactivity and a low molecular complexity may result in an expanded therapeutic window which will enable further clinical evaluation of synthetic enediynes.

Although our results suggest that the usefulness of established tumor cell lines to predict in vivo responses to enediyne therapy may be suspect, the antitumor activity of enediyne 1 makes identification of the enediyne target and its mechanism of induction of cell death a clear priority. Cells in culture must acquire or be selected for expression of new capabilities in order to proliferate as tumors. If there is a common target of enediyne activity both in cultured cells and in vivo, it may provide a useful biochemical marker to begin to understand the process of adaptation of tumor cells in living organisms.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant CA46446-0651 and by the Scripps Research Institute.

REFERENCES

1. Lee MD, Dunne TS, Siegel MM, Chang CC, Morton GO, Borders DB. Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin γ1. J Am Chem Soc 1987; 109: 3464–6.
2. Lee MD, Dunne TS, Chang CC, et al. Calichemicins, a novel family of antitumor antibiotics. 2. Chemistry and structure of calichemicin γ1. J Am Chem Soc 1987; 109: 3466–8.
3. Golik J, Clardy J, Dubay G, et al. Esperamicins, a novel class of potent antitumor antibiotics. 2. Structure of esperamicin X. J Am Chem Soc 1987; 109: 3461–2.
4. Golik J, Dubay G, Groenewold G, et al. Esperamicins, a novel class of potent antitumor antibiotics. 3. Structures of esperamicin-A1, esperamicin-A2, and esperamicin A1B. J Am Chem Soc 1987; 109: 3462–6.
5. Ishida N, Miyazaki K, Kumagai K, Rikimaru M. Neocarzinostatin, an antitumor antibiotic of high molecular weight. J Antibiot 1965; 18: 68–76.
6. Napier MA, Holmquist B, Strydom DJ, Goldberg IH. Neocarzinostatin—spectral characterization and separation of non-protein chromophore. Biochem Biophys Res Commun 1979; 89: 635–42.
7. Konishi M, Ohkuma H, Matsumoto K, et al. Dynemicin A, a novel antibiotic with the anthraquinone and 1,5-diyn-3-ene subunit. J Antibiot 1989; 42: 1449–53.
8. Suzuki H, Miura K, Kumada K, Taeuchi T, Tanake N. Biological activities of non-protein chromophores of antitumor protein antibiotics: auromycin and neucarzinostatin. Biochem Biophys Res Commun 1980; 94: 255–61.
9. Konishi M, Ohkuma H, Tsuno T, Oki T. Crystal and molecular structure of dynemicin A: A novel 1,5-diyn-3-ene antitumor antibiotic. J Am Chem Soc 1990; 112: 3715–6.
10. Leet JE, Schroeder DR, Hofstead SJ, et al. Kedarcidin, a new chromoprotein antitumor antibiotic: structure elucidation of kedarcidin chromophore. J Am Chem Soc 1992; 114: 7946–8.
11. Bergman RG. Reactive 1,4-dehydroaromatics. Acc Chem Res 1973; 6: 25–32.
12. Jones RR, Bergman RG. p-Benzyne, generation as an intermediate in a thermal isomerization reaction and trapping evidence for the 1,4-benzenediyne structure. J Am Chem Soc 1972; 94: 660–3.
13. Povirk LF, Goldberg LH. Competition between anaerobic covalent linkage of neocarzinostatin chromophore to deoxyribose in DNA an oxygen-dependent strand breakage and base release. Biochemistry 1984; 23: 6304–11.
14. Long BH, Golik J, Forenza S, et al. Esperamicins, a class of potent antitumor antibiotics. Mechanism of action. Proc Natl Acad Sci USA 1989; 86: 2–6.
15. Zein N, Sinha AM, McGahren WJ, Ellestad GA. Calicheamicin γ_1^1: an antitumor antibiotic that cleaves double-stranded DNA site specifically. Science 1988; 240: 1198–201.
16. Sugiura Y, Shiraki T, Konishi M, Oki T. DNA intercalation and cleavage of an antitumor antibiotic dynemicin that contains anthracyline and enediyne cores. Proc Natl Acad Sci USA 1990; 87: 3831–5.
17. Koide W, Ishii F, Kasuda K, et al. Isolation of a non-protein component and a protein component from neocarzinostatin (NCS) and their biological activities. J Antibiot 1980; 33: 342–8.
18. Maiese WM, Lechevalier MP, Lechevalier HA, et al. Calicheamicins, a novel family of antitumor antibiotics: taxonomy, fermentation, and biological properties. J Antibiot 1989; 4: 558–63.
19. Nicolaou KC, Dai W-M. Chemistry and biology of the enediyne anticancer antibiotics. Angew Chem Int Engl 1991; 30: 1387–416.
20. Nicolaou KC, Dai W-M, Tsay S-C, Estevez VA, Wrasidlo W. Designed enediynes: a new class of DNA-cleaving molecules

with potent and selective anticancer activity. Science 1992; 256: 1172–8.
21. Nicolaou KC, Stabila P, Esmaeli-Azad B, Wrasidlo W, Hiatt A. Cell specific regulation of apoptosis by designed enediynes. Proc Natl Acad Sci USA 1993. (in press.)
22. Nicolaou KC, Maligres P, Suzuki T, Wenderborn SV, Dai W-M, Chadha RK. Molecular design and chemical synthesis of potent enediynes. 1. Dynemicin model systems equipped with N-tethered triggering devices. J Am Chem Soc 1992; 114: 8890–907.
23. Myers C, Cooper M, Stein C, et al. Suramin: a novel growth factor antagonist with activity in hormone-refractory metastatic prostate cancer. J Clin Oncol 1992; 10: 875–7.
24. Szende B, Srkalovic G, Groot K, Lapis K, Schally AV. Regression of nitrosamine-induced pancreatic cancers in hamsters treated with luteinizing hormone-releasing hormone antagonists or agonists. Cancer Res 1990; 50: 3716–21.
25. Trauth BC, Klas C, Peters AMI, et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science 1989; 243: 301–4.
26. Kyprianou N, English HF, Isaacs JT. Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation. Cancer Res 1990; 50: 3748–53.
27. Kyprianou N, English HF, Davidson NE, Isaacs JT. Programmed cell death during regression of the MCF-7 human breast cancer following estraogen ablation. Cancer Res 1991; 52: 162–6.