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Cellular resistance to the antimelanoma immunotoxin ZME-gelonin and strategies to target resistant cells

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Abstract The development of cellular resistance to immunotoxins has been demonstrated in a variety of models and can involve a number of mechanisms. For the present study, an immunotoxin was utilized composed of an antimelanoma antibody ZME-018 recognizing a 240-kDa surface glycoprotein (gp 240) and the plant toxin gelonin. Human melanoma cells (A375-M) were grown in the presence of increasing amounts of ZME-gelonin and a clonal variant (A-375-ZR) was developed that was 100-fold resistant to ZME-gelonin compared to parental cells. Scatchard analysis showed that the A375-M parental cells had 260×10^3 ZME-gelonin-binding sites/cell with relatively low affinity (5 nM). In contrast, resistant A375-ZR cells demonstrated a reduced number of low-affinity sites (160×10^3/cell), but showed a small number (47×10^3) of higher-affinity sites (0.8 nM). Internalization rates and degradation rates of ^{125}I-labeled ZME-gelonin were identical in both the parental and resistant cells. A375-ZR cells were found to be more resistant to vincristine and doxorubicin than were parental cells. Both cell lines were almost equally sensitive to native gelonin, 5-fluorouracil (5-FU), cisplatin, melphalan, carmustine, interferon γ (IFNγ) and IFNα. In addition, both cell lines were equally sensitive to another gelonin-antibody conjugate that binds to cell-surface, GD_2 (antibody $14G_2A$). However, resistant cells were twice as sensitive to the cytotoxic effects of etoposide than were parental cells. Finally, a variety of agents were tested in combination with ZME-gelonin against A375-ZR cells in an attempt to identify agents to augment immunotoxin cytotoxic effects against resistant cells. The agents 5-FU, cisplatin, IFNγ, IFNα, and etoposide were the most effective in augmenting the cytotoxicity of ZME-gelonin against resistant cells. These studies suggest that development of resistance to one immunotoxin does not cause development of cross-resistance to other gelonin immunotoxins. Further, specific biological response modifiers and chemotherapeutic agents may be effective in augmenting the effectiveness of immunotoxins and specifically targeting or reducing the emergence of immunotoxin-resistant cells.

Key words Gelonin · Immunotoxins · Chemotherapeutic agents · Drug resistance · Melanoma

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Introduction

Lack of response to radiotherapy and cytotoxic chemotherapy is the major cause of treatment failure in patients with cancer. Studies of the emergence of cellular resistance to therapeutic agents is exceptionally important to understanding both the biology of neoplastic disease and methods for successful therapeutic intervention. The development of iatrogenic or innate resistance to chemotherapeutic agents has been under study for many years and there are a variety of biochemical mechanisms identified that contribute to the development of resistance at the cellular level [4, 5, 10, 12, 19, 23]. Antibody-guided drugs and toxins are also susceptible to this shortcoming since resistance to antibody-toxin immunoconjugates has been shown to exist and may occur through a variety of mechanisms. Several investigators [16, 7, 33] have demonstrated that some human tumor cells resistant to immunotoxins lack cell-surface antigens targeted by the antibody, resulting in outgrowth of antigen-negative cells. Wargalla and Reisfeld [34] have shown that cytotoxicity of immunotoxins against melanoma cells may be primarily regulated by the rate of cellular internalization and processing of the intracellular complex. In contrast, recent studies by May et al. [18] suggest that it is the intracellular trafficking of immunotoxins which is a predominant determinant of cytotoxicity.

The purpose of this study was to develop clonal variants of human melanoma cells resistant to antibody-toxin conjugates and to examine the characteristics associated with resistance developed in vitro. A second objective was to identify chemotherapeutic or biological agents that may be useful in overcoming or modifying cellular resistance to immunotoxins. These studies are important for the identification of rational approaches to combining immunotoxins with conventional therapeutic agents. Combination treatment may augment and extend a therapeutic advantage over immunotoxins as single agents.

Materials and methods

Materials

Antibody ZME-018 was kindly provided by Hybritech Inc. (San Diego, Calif.) and purified using salt fractionation and DEAE chromatography. Murine antibody $14G_2A$ was a generous gift of Dr. Ralph Reisfeld, Scripps Clinic and Research Foundation, La Jolla, Calif. Gelonin seeds were purchased from United Chemicals and Allied Products (Calcutta, India). Doxorubicin was purchased from Adria Laboratories, Dublin, Ohio. 5-fluorouracil was purchased from Solopak Labs, Franklin Park, Ill. Cisplatin (*cis*-diaminedichloroplatinum II), carmustine and etoposide were purchased from Bristol-Myers/Squibb, Syracuse, N.Y. Vincristine, leupeptin, amantadine, propylthiouracil, verapamil, colchicine, bacitracin, mannose 6-phosphate, nigericin and monensin were obtained from Sigma Chemical Co., St. Louis, Mo. Melphalan was purchased from Burrows, Wellcome, Research Triangle Park, N.C. Interferon α (IFNα) (sp.act. 2×10^8 U/mg) and IFNγ (sp.act. 2×10^7 U/mg) were generous gifts of Roche Laboratories, Nutley, N.J., and Genentech Inc., South San Francisco, Calif., respectively.

Preparation of ZME-gelonin and $14G_2A$-gelonin conjugates

Both ZME and $14G_2A$ were covalently coupled to the purified plant toxin gelonin as previously described [21, 26, 30]. Tests to confirm immunotoxin binding to target cells, specificity and in vitro cytotoxicity were performed and evaluated as described previously. Radiolabeling of the immunotoxin was also performed as previously described [22].

Cell culture methods

The parental and clonal variant lines of A375-M human melanoma were maintained in culture using minimal essential medium (Gibco, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, Utah) plus non-essential amino acid supplement (Gibco), 2 mM L-glutamine (Gibco), 1 mM sodium pyruvate, and 50 μg/ml gentamicin (Tri-Bio Labs). Cells were subcultured once weekly and were routinely screened and found free of *Mycoplasma* infection.

Cell proliferation assay

For assays with combinations of immunotoxins and other agents, cell monolayers were washed with phosphate-buffered saline (PBS), detached using versene and resuspended in complete medium at a density of 5×10^4 cells/ml. Aliquots of 100 μl were dispensed into 96-well microtiter plates and the cells were then allowed to attach overnight. After 24 h the medium was replaced with an appropriate concentration of either immunotoxin, or other chemotherapeutic agents in complete medium. The cells were incubated for 72 h and analyzed for relative cell proliferation by crystal violet staining as previously described [3].

Isolation of a ZME-gelonin-resistant cell line

Log-phase A375-M parental cells were treated with increasing doses (up to 40 μg/ml) of purified ZME-gelonin. Surviving cells were harvested, maintained for 2 weeks in ZME-gelonin and cloned by limiting dilution in the presence of ZME-gelonin. Colonies growing in the presence of ZME-gelonin were expanded and grown in the absence of immunotoxin and then re-tested for sensitivity to immunotoxin. One clonal population designated A375-ZR was chosen for further study.

ELISA assay of ZME-gelonin on sensitive and resistant cells

ELISA assays were performed to determine the immunoreactivity of the ZME-gelonin conjugate on A375-M (parental) and A375-ZR (resistant) cells. Briefly, 5×10^4 melanoma cells (A375-M or A375-ZR) were added to individual wells of a 96-well polyvinyl microtiter plate (Falcon). The plates were dried for 18 h at 37 °C and then washed twice with 10 mM PBS, pH 7.4, containing 0.1% Tween-20 and 0.02% Thimersol (washing buffer). ZME-gelonin conjugate was diluted in washing buffer containing 0.1% bovine serum albumin, added to each well and plates were incubated for 1 h at room temperature. After three washes with washing buffer, horseradish-peroxidase-conjugated goat anti-(mouse IgG) (BioRad) was added to each well and incubated for 1 h at room temperature. After three washes, 100 μl 1 mM 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) containing 0.03% H_2O_2 was added to each well. The reaction was stopped after 10–20 min by addition of 5% sodium dodecyl sulfate (SDS) in PBS and absorbance at 405 nm was measured with an enzyme-linked immunosorbent assay (ELISA) reader.

Determination of total cell binding and affinity constant (K_a)

Melanoma cells (10^5 of A375-M and A375-ZR) were plated in 24-well plates and allowed to adhere overnight at 37 °C. The cells were washed with ice-cold medium and incubated with various concentrations (1–16 nM) of ^{125}I-labeled ZME-gelonin immunotoxin for 4 h at either 4 °C or 37 °C. The non-specific binding was determined by measuring immunoconjugate binding in the presence of a 100-fold molar excess of unlabelled antibody. Cells were washed three times with ice-cold medium containing 1% bovine serum albumin and lysed with 0.1 M NaOH containing 0.1% SDS. All analyses were performed in duplicate and quantified in a gamma scintillation counter. Data were analyzed by the method of Scatchard [27] to determine the affinity constant and number of binding sites.

Determination of the internalization rate constant (K_e)

The method has been previously described elsewhere [10]. Briefly, 2 nM ^{125}I-labeled ZME-gelonin immunotoxin was added to A375-M (parental) and A375-ZR (resistant) melanoma cells for up to 4 h at 4 °C or 37 °C. Aliquots of binding mixtures were removed every 30 min and cell-surface ligand was distinguished from internalized ligand by treating the cells with 1 ml ice-cold 0.2 M acetic acid (pH 2.5) containing 0.5 M NaCl for 8 min at 0 °C. After two final washes with ice-cold medium, the remaining cell-associated radioactivity was removed by treating cell monolayers with 1 M NaOH (pre-warmed to 60 °C). Internalized and cell-associated ^{125}I-labeled ZME-gelonin were determined by γ counting. Non-specific binding was approximately 10% of total binding and was subtracted from each data point. K_e of ^{125}I-ZME-gelonin, which defines the probability of an occupied receptor (antigen) being internalized in 1 min at 37 °C, was determined according to Wiley and Cunningham [35]. This was analyzed by applying the "in/sur" (ratio of internalized to surface-bound ZME-gelonin) plot technique to estimate the internalization rate constant (K_e).

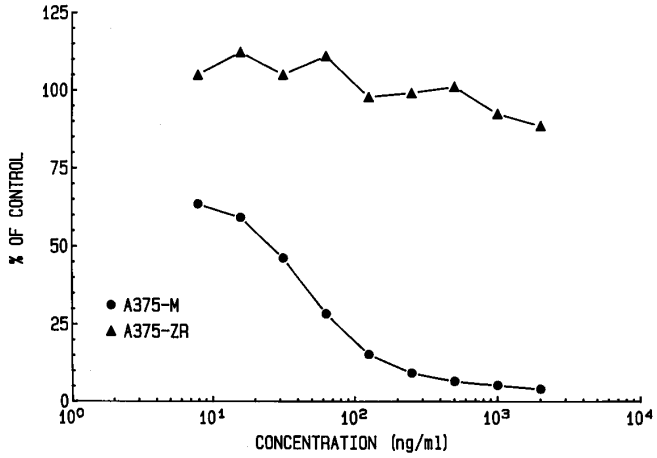

Fig. 1 Antiproliferative effects of ZME-gelonin on A375-M human melanoma cells (●-●) and A375-ZR immunotoxin-resistant (▲-▲) cells. Various concentrations of immunotoxin were added to log-phase cells and incubated for 72 h at 37 °C. Viable cell number was then assessed using crystal violet stain. Values represent the percentage cytotoxicity based upon control growth. This figure is representative of the different experiments performed in octuplicate

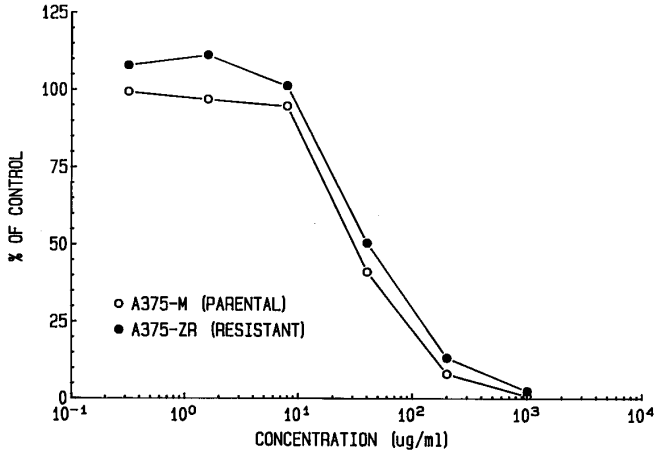

Fig. 2 Antiproliferative effects of gelonin on A375-M human melanoma cells (○-○) and A375-ZR immunotoxin resistant (●-●) cells. Various concentrations of gelonin were added to log-phase cells and incubated for 72 h at 37 °C. Viable cell number was then assessed using crystal violet stain. Values shown are representative of three experiments performed in octuplicate

Determination of metabolism of ^{125}I-ZME-gelonin by sensitive and resistant cells

The extent of degradation of ^{125}I-ZME-gelonin on A375-M and A375-ZR cells was measured as previously described by Press et al. [25]. Briefly, A375-M and A375-ZR cells were seeded in six-well plates at a density of 0.5×10^6 cells/well. The cells were incubated at 37 °C overnight, an aliquot of 0.5 μCi ^{125}I-ZME-gelonin in 2 ml complete medium was added to each well and the plates were further incubated at 37 °C or at 4 °C for 1 h. Plates were then washed twice with ice-cold medium, fresh medium was added and plates were then incubated at 37 °C for 0, 1, 4 or 24 h. Culture supernatants (0.2 ml) were mixed with 0.6 ml 25% trichloroacetic acid (Cl_3AcOH) to precipitate protein-bound ^{125}I released from the cell surface. Precipitates were washed twice with 0.5 ml 25% Cl_3AcOH and the radioactivity in the pellets (Cl_3AcOH-insoluble) and supernatants (Cl_3AcOH-soluble) was determined.

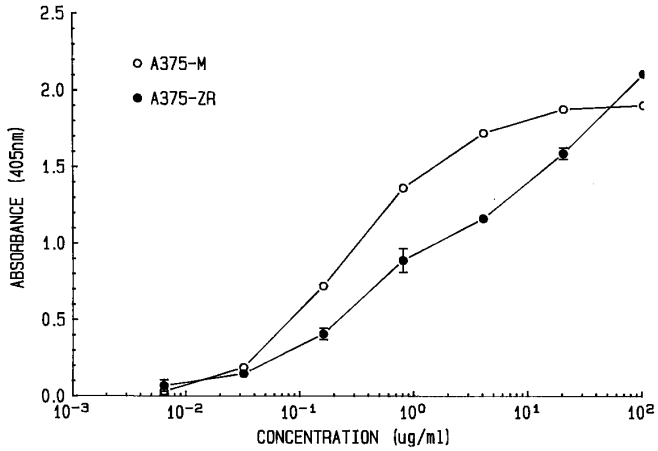

Fig. 3 Comparative ELISA assay of ZME-gelonin immunotoxin on A375-M parental (○) and A375-ZR resistant (●) cells. Various concentrations of immunotoxin were added and incubated at room temperature for 60 min. The cells were then washed and a standard ELISA was performed. Values shown are means from two experiments performed in octuplicate

Results

Development of an immunotoxin-resistant cell line

Log-phase human A375-M cells were grown in the presence of increasing concentrations of ZME-gelonin immunotoxin. Clonal populations obtained by limiting dilution were found to maintain resistance to ZME-gelonin after several passages in the absence of immunotoxin. One clone, designated A375-ZR, was found to be resistant to ZME-gelonin at concentrations exceeding 1 μg/ml. As shown in Fig. 1, the inhibitory concentration of ZME-gelonin on A375-M parental cells was approximately 20 ng/ml while doses of 2000 ng/ml resulted in only 15%–20% growth inhibition of the A375-ZR cells. The A375-ZR line demonstrated identical morphological characteristics and doubling times (22 h) compared to the parental cell line (18 h).

While the cytotoxic effects of ZME-gelonin on these two cell lines were quite different, both lines responded equally to the cytotoxic effects of native gelonin (Fig. 2) suggesting that the core mechanisms of protein synthesis inhibition by interaction of the toxin at the ribosomal subunit was similar in the two cell lines.

Cell surface and internalization studies

A possible mechanism of cellular immunotoxin resistance could be reduction of immunotoxin binding due to the expansion of an antigen-negative sub-population [32, 33]. To investigate this possibility, an ELISA assay was first performed to determine whether gross changes occur in the binding of ZME-gelonin to the A375-ZR cells. As shown in

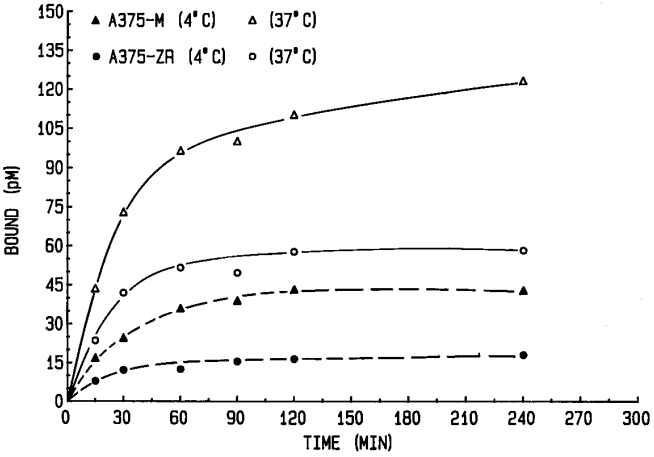

Fig. 4 Immunoreactivity of ZME-gelonin immunotoxins on A375-M human melanoma parental and A375-ZR resistant cells. Various concentrations (1–16 mM) of ^{125}I-labeled ZME-gelonin were added and incubated for up to 4 h either at 4 °C (●, ▲) or at 37 °C (○, △); ▲, △ A375-M, ●, ○ A375-ZR. Values shown are means from two experiments performed in duplicate

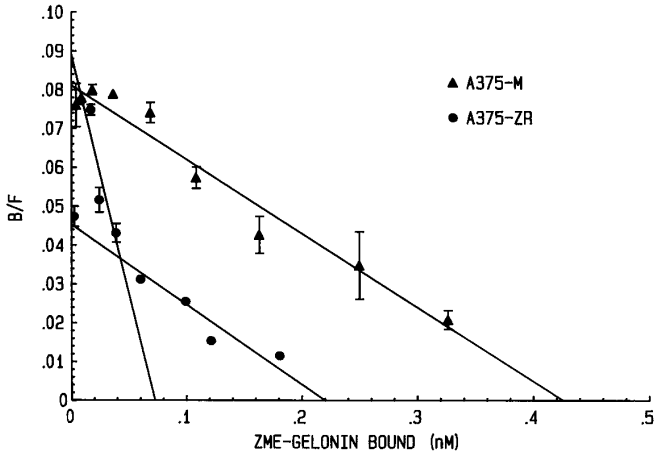

Fig. 5 Scatchard plot analysis of ZME-gelonin immunotoxins on A375-M parental (▲) and A375-ZR resistant (●) cells. This figure shows that two classes of affinity site were presented on the immunotoxin-resistant (A375-ZR) cells, whereas a single, homogeneous class of affinity sites was found on the human melanoma parental (A375-M) cells. Values shown are the means from two experiments performed in triplicate

Fig. 3, the binding of ZME-gelonin to resistant cells paralleled that of the parental cell line. However, total binding to the A375-ZR cell line appeared to be reduced compared to that of the parental cells.

Binding studies with ^{125}I-labeled ZME-gelonin performed at 4 °C and 37 °C (Fig. 4) demonstrated that the A375-ZR cells had approximately a 2.5-fold lower specific binding capacity compared to the A375-M parental cultures. Scatchard analysis of ^{125}I-ZME-gelonin binding (Fig. 5, Table 1) demonstrated that the parental A375-M cells bound the immunotoxin to a single class of receptor with a density of approximately 260 000 sites/cell and an affinity constant of 5 nM. Analysis of ^{125}I-ZME-gelonin binding to the resistant cell line demonstrated two classes of affinity sites: a low-affinity site (K_d = 5.9 nM) similar to that found on the parental cells but with a lower number of receptors (160 000 sites/cell compared to 260 000 sites/cell). In addition, a relatively small number (47 000 sites/cell) of high-affinity sites (0.78 nM) were measured on the resistant cell line.

The rate of internalization of immunotoxin has also been reported to be of significance in the modulation of cytotoxicity of immunotoxins. Studies performed on A375-M (parental) and A375-ZR (resistant) cells (Fig. 6) suggested that the apparent internalization rate of ZME-gelonin immunotoxin was identical in both parental and resistant cell lines.

To determine whether gross changes in the metabolism of immunotoxins play a role in, or account for cellular resistance, the metabolism of ^{125}I-ZME-gelonin was next studied. As shown in Fig. 7, approximately 30% of the radiolabel was degraded within the first 5 h of incubation. Approximately 20% more degradation occurred over the next 19 h (about 50% total degradation over 24 h). Incubation of cells with ^{125}I-ZME-gelonin at 4 °C for 1 h initially slowed the degree of degradation of immunotoxin in the first 90 min. However, total degradation of immunotoxin in cells treated for 24 h at 37 °C was identical to that of cells maintained at 4 °C. Therefore, although the rates appear to

Table 1 Scatchard analysis of immunotoxin-sensitive and -resistant melanoma cell lines

Cells	10^{-3} Receptor number	K_d (nM)
A375-M	260	5.00
A375-ZR		
High	47	0.78
Low	160	5.9

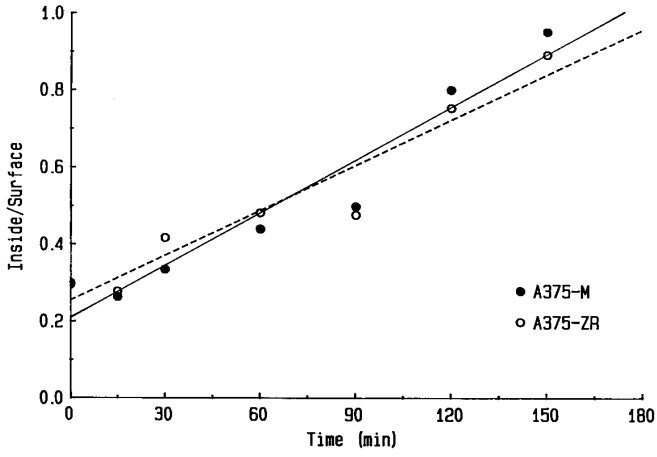

Fig. 6 Rate of internalization of ZME-gelonin immunotoxins on A375-M human melanoma parental (●) and resistant cell (○) cells. A plot of the ratio of internalized to surface-bound ^{125}I-ZME-gelonin. Values shown are the means from three experiments performed in duplicate

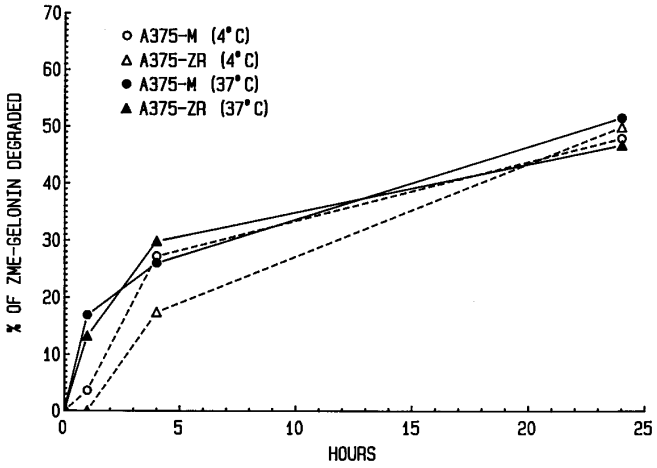

Fig. 7 Degradation of ZME-gelonin immunotoxin on A375-M human melanoma parental (○, ●) and resistant (△, ▲) cells. An aliquot of 0.5 μCi ^{125}I-labeled ZME-gelonin immunotoxin was added for 1 h at either 4 °C (○, △) or at 37 °C (●, ▲). The plates were then washed twice and incubation continued for up to 24 h. Values shown are the means from two separate experiments performed in duplicate

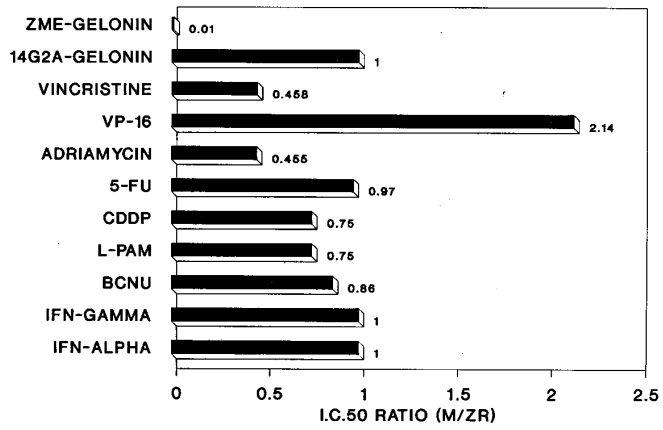

Fig. 8 Effect of various biological response modifiers and chemotherapeutic agents on the A375-M human melanoma (parental and resistant) cells. The figure demonstrates the ratio of IC_{50} of each of the drugs on parental and resistant cells. Values shown are the results of three separate experiments each performed in octuplicate. *VP-16* etoposide, *ADRIAMYCIN* doxorubicin; *5-FU* 5-fluorouracil, *CDDP* cisplatin, *L-PAM* melphalan, *BCNU* carmustine, *IFN* interferon

be distinct, overall the cellular degradation processes for immunotoxins appear to be temperature-independent.

Sensitivity of A375-M and A375-ZR cells to chemotherapeutic agents and biological response modifiers

The cytotoxic effects of various agents alone on the ZME-gelonin-sensitive and -resistant cell lines were next compared to determine whether development of phenotypic resistance to immunotoxins has an effect on specific cellular responses to various agents. Dose response curves for the various classes of therapeutic agents were analyzed and the concentrations required to inhibit cells to 50% of the control values were calculated (IC_{50}). The ratios of IC_{50} values for the parental line compared to the A375-ZR line are shown in Fig. 8. As described previously, A375-M cells were approximately 100-fold more sensitive than the A375-ZR line to ZME-gelonin immunotoxin. Both cell lines exhibited almost equivalent sensitivity to 5-FU, carmustine and both IFNα and γ. The immunotoxin-resistant cells were slightly more resistant to the alkylating agents L-phenylalanine mustard (melphalan) and platinum complexes (cisplatin) than the parental cells. The A375-ZR cells demonstrated the greatest cross-resistance to vincristine and doxorubicin when compared to parental cells. However, the cytotoxic effects of 14G2a-gelonin immunotoxin were identical on both sensitive and resistant cell lines suggesting that cellular resistance to one antibody-driven toxin does not predict resistance to other immunotoxins. A375-

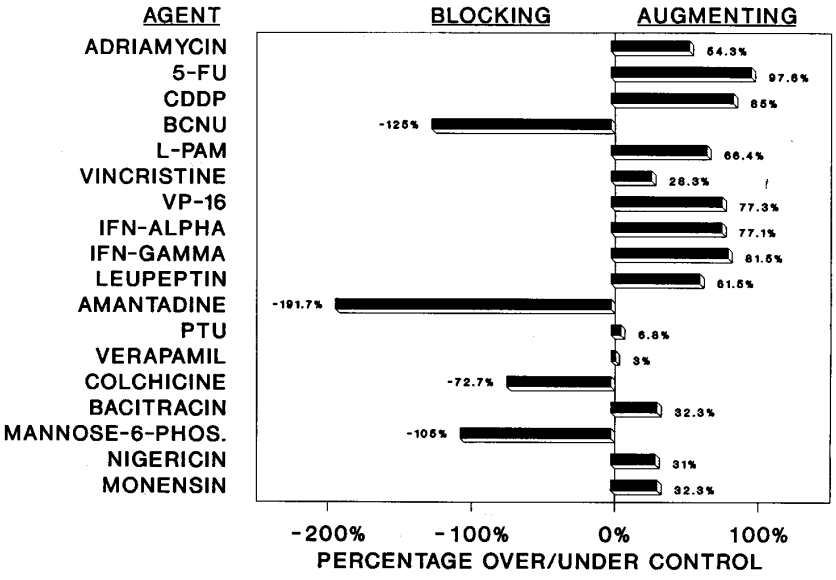

Fig. 9 Effect of various biological response modifiers and chemotherapeutic agents in combination with ZME-gelonin immunotoxin on A375-ZR human melanoma immunotoxin-resistant cells. A fixed dose of individual agents that caused up to 10% cell growth inhibition alone was added to various concentrations of ZME-gelonin on A375-ZR cells. The percentage change in the IC_{50} dose with and without each agent was then calculated. Values shown are the results from three experiments each performed in octuplicate. Abbreviations as in Fig. 8

ZR cells were more than twice as sensitive to etoposide than were the parental cells.

A variety of agents were then tested in combination with ZME-gelonin against resistant A375-ZR cells to identify the agents that may be capable of circumventing or reversing immunotoxin resistance. As shown in Fig. 9, the most effective agents for potentiating ZME-gelonin activity against resistant cells were 5-FU, cisplatin, IFNγ, IFNα and etoposide. Less effective augmenting effects were seen with doxorubicin, the alkylating agent melphalan and the lysosomal enzyme inhibitor leupeptin. The agents vincristine, propylthiouracil, verapamil and colchicine had little or no effect on the cytotoxic action of ZME-gelonin against resistant cells. In contrast, the alkylating agent carmustine, the lysosomotropic amine amatadine and mannose 6-phosphate all exacerbated the resistance of A375-ZR cells to the cytotoxic effects of ZME-gelonin.

Discussion

Passive serotherapy with murine monoclonal antibodies has shown limited antitumor activity in a variety of phase I clinical trials [28, 11]. To improve their therapeutic efficacy, antibodies have been conjugated to a variety of toxins, drugs and radioisotopes [13, 31]. Monoclonal antibodies linked to potent plant or bacterial toxins such as the A chain of ricin, gelonin or diphtheria toxin have shown variable efficacy in some in vitro cytotoxicity tests as well as variable in vivo efficacy against transplanted tumors. Factors such as the cell-surface antigen target, antigen density, antibody affinity, immunotoxin construction and rates of immunoconjugate internalization have all been proposed to affect the cytotoxic efficacy of immunotoxins [14, 24].

While the sensitivity of different cell lines to immunotoxins may vary because of modulation of a number of properties described above, the development of cellular resistance to immunotoxins has, in general, been shown to be multi-factorial [1, 20] Lesley et al. [16] have shown that resistance of human lymphoma cells to a ricin-containing immunotoxin appears to be due to the emergence of antigen-negative clonal variants. On the other hand, studies by Kornfield [15] have suggested that intracellular trafficking of ricin A chain immunotoxins may also play an important role in the emergence of cellular resistance to these conjugates. The current study demonstrates that development of resistance to a gelonin immunotoxin may be the result of an interplay of several factors. While the affinity for the cellular ligand in resistant cells appeared similar to that of sensitive cells, the absolute number of antigen sites was substantially reduced. However, internalization rates of immunotoxin were shown to be identical for both sensitive and resistant cell lines. One observation in this study was that the development of resistance to ZME-gelonin did not appear to influence the inherent ability of the gelonin toxin itself to affect both sensitive and resistant cells. Therefore, the ability of the antibody ZME to deliver active toxin successfully into the appropriate subcellular compartment may be altered in the resistant cell line. One possible explanation may be the apparent development of a small subset of high-affinity cell-surface ligands for ZME described only in the resistant cell line. It is possible that these sites may reroute cellular trafficking of ZME-gelonin so that active toxin is prevented from reaching the sensitive cellular compartment.

The use of various lysosomotropic agents to overcome immunotoxin resistance or to augment cellular sensitivity to immunotoxins has been reported by several investigators [2, 6]. However, in this study, the use of the lysosomotropic amines amatadine, nigericin and monensin were unable to augment the response of resistant cells to ZME-gelonin substantially. These results suggests that modulation of the lysosomal compartment may not play a major role in the development of resistance to gelonin-based immunotoxins in this cell line. Surprisingly, mannose 6-phosphate, which has been shown to enhance antibody internalization [9], appeared to suppress the activity of ZME-gelonin 2-fold. While immunotoxin internalization was not measured in the presence of mannose 6-phosphate, one possible explanation for this observation may be that internalization of the immunotoxin could be augmented but shunted to an intracellular compartment without access to the ribosomal fraction. An alternative explanation is that mannose 6-phosphate may prevent binding of ZME-gelonin to the cell surface. Investigation into the two hypotheses are currently underway.

Using an antimelanoma antibody (9.2.27) conjugated to pokeweed antiviral protein (PAP), Morgan et al. [29] have shown that immunotoxin-resistant human melanoma cells demonstrate no change in antigen expression. These data appear to concur with the current study. However, melanoma cells resistant to 9.2.27-PAP were found to be cross-resistant to conjugates of 9.2.27 and other A-chain toxins. In a similar study of human β cell (Namalwa) clones resistant to an anti-CALLA antibody-ricin conjugate, Goldmacher et al. [7], found that cells did not appear to be cross-resistant to antibody anti-CALLA conjugates utilizing gelonin. The ability of 14G2a-gelonin toxin to affect ZME-gelonin-resistant and -sensitive cells equally in this study suggests that resistance of cells to one antibody-driven gelonin toxin does not have a universal effect on other antibody-driven gelonin toxins. Therefore, cycling immunotoxin therapy between two or more different immunotoxins may be an important strategy in preventing the emergence of immunotoxin-resistant cells.

The increased sensitivity of A375-ZR cells to one specific class of chemotherapeutic agents, etoposide, is also important to note for a variety of reasons. The mechanism of podophyllotoxin cytotoxic effects involve both inhibition of tubular polymerization and modulation of DNA topoisomerase-II activity leading to protein-linked DNA strand breaks. The increased sensitivity of A375-ZR cells to etoposide may suggest that development of resistance to immunotoxins may either require or result in modulation of DNA-topoisomerase-II-dependent biochemical events or may influence tubulin polymerization [17]. Additionally, agents such as etoposide were found to be

some of the most active in augmenting the response of resistant cells to ZME-gelonin. Therefore, these data indicate that the combination of a ZME-gelonin therapeutic regimen with etoposide may both augment immunotoxin cytotoxicity and prevent development of cellular resistance. Studies are ongoing to ascertain the utility of in vivo combinations of ZME-gelonin immunotoxin with various chemotherapeutic agents and with other gelonin-based immunotoxins.

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