In vitro and In vivo Selective Antitumor Activity of Edelfosine against Mantle Cell Lymphoma and Chronic Lymphocytic Leukemia Involving Lipid Rafts

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Abstract

Purpose: Mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) remain B-cell malignancies with limited therapeutic options. The present study investigates the in vitro and in vivo effect of the phospholipid ether edelfosine (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine) in MCL and CLL.

Experimental Design: Several cell lines, patient-derived tumor cells, and xenografts in severe combined immunodeficient mice were used to examine the anti-MCL and anti-CLL activity of edelfosine. Furthermore, we analyzed the mechanism of action and drug biodistribution of edelfosine in MCL and CLL tumor-bearing severe combined immunodeficient mice.

Results: Here, we have found that the phospholipid ether edelfosine was the most potent alkyl-lysophospholipid analogue in killing MCL and CLL cells, including patient-derived primary cells, while sparing normal resting lymphocytes. Alkyl-lysophospholipid analogues ranked edelfosine > perifosine > erucylphosphocholine ≥ miltefosine in their capacity to elicit apoptosis in MCL and CLL cells. Edelfosine induced co-clustering of Fas/CD95 death receptor and rafts in MCL and CLL cells. Edelfosine was taken up by malignant cells, whereas normal resting lymphocytes hardly incorporated the drug. Raft disruption by cholesterol depletion inhibited drug uptake, Fas/CD95 clustering, and edelfosine-induced apoptosis. Edelfosine oral administration showed a potent in vivo anticancer activity in MCL and CLL xenograft mouse models, and the drug accumulated dramatically and preferentially in the tumor.

Conclusions: Our data indicate that edelfosine accumulates and kills MCL and CLL cells in a rather selective way, and set co-clustering of Fas/CD95 and lipid rafts as a new framework in MCL and CLL therapy. Our data support a selective antitumor action of edelfosine. Clin Cancer Res; 16(7); 2046–54. ©2010 AACR.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) are two major B-cell-derived neoplasias for which current therapy is not satisfactory, leading in most cases to relapse and eventually to a fatal outcome. This lack of efficient therapy underscores the need for a continued search for novel chemotherapeutic agents. CLL is the most common adult leukemia and is characterized by the progressive accumulation of mature CD5+ B lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs. New treatment combinations have incorporated the use of purine analogue (fludarabine)-based regimens together with monoclonal antibodies rituximab (anti-CD20) and alemtuzumab (anti-CD52), leading to improved complete response rates and prolonged progression-free survival, but a long-term survival benefit has not been shown (1, 2). MCL is characterized by the chromosomal translocation t(11;14)(q13;q32), resulting in the overexpression of cyclin D1 in mature B
Mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) lack effective therapy. Synthetic alkyl-lysophospholipid analogues constitute a family of promising anticancer drugs, including miltefosine, perifosine, edelfosine, and erucylphosphocholine, which promote apoptosis in a variety of tumor cells. Here, we have found that edelfosine behaves as the most potent alkyl-lysophospholipid analogue in inducing cell death in MCL and CLL cells through co-clustering of Fas/CD95 and rafts. Edelfosine induced a higher apoptotic response than perifosine in MCL and CLL patient-derived cells. Oral administration of edelfosine showed a strong in vivo anti-MCL and anti-CLL activity in xenograft mouse models. The drug accumulated in a dramatic and preferential way in the tumor, leading to drastic tumor regression. Our data reported here show a rather selective action of edelfosine against tumor cells and provide the proof of principle and rationale for further clinical evaluation of edelfosine to improve patient outcome in MCL and CLL.

**Materials and Methods**

**Drugs.** Edelfosine was from Inkeysa and Apointech. Miltefosine was from Calbiochem. Perifosine and erucylphosphocholine were from Zentaris.

**Cell lines and primary cells.** Detailed information on the cell culture conditions for human MCL (JVM-2 and Z-138) and CLL (EHEB) cell lines and primary cells from CLL and MCL patients is included in Supplementary Data.

**Apoptosis assay.** Quantitation of apoptotic cells was determined by flow cytometry as the percentage of cells in the sub-G1 region (hypodiploidy) in cell cycle analysis as previously described (26).

To analyze apoptosis in CLL/MCL patient-derived samples, 5 x 10^6 cells were incubated for 48 h with the indicated agents. Cells were then washed in Annexin-binding buffer and incubated in 50 μL Annexin-binding buffer with allophycocyanin-conjugated anti-CD3 and phycoerythrin-conjugated anti-CD19 antibodies from Becton Dickinson for 10 min in the dark. Cells were then diluted with Annexin-binding buffer to a volume of 150 μL and incubated with 1 μL FITC-labeled Annexin V (Becton MedSystems) for 15 min in the dark. A total of 10,000 stained cells were then analyzed by flow cytometry on a FACSCalibur flow cytometer using CellQuest software (Becton Dickinson).

**Western blot.** Proteins (50 μg) were separated on 12% SDS-PAGE and transferred onto Immobilon-P membranes (Millipore). Membranes were probed with anticyclin D1 (DCS-6, Cell Signaling Technology) and anti-β-actin (Sigma) antibodies. Antibody binding was detected using the enhanced chemiluminescence detection system (Amersham).

**Confocal microscopy.** Cells were settled onto poly-l-lysine–coated slides and analyzed with a Zeiss LSM 510 laser scan confocal microscope for membrane raft and Fas/CD95 visualization using FITC-labeled cholera toxin B subunit (Sigma) and anti-human Fas/CD95 SM1/1 IgG2a mouse monoclonal antibody (Bender MedSystems).
followed by CY3-conjugated anti-mouse antibody (Phar- 
macia), as described (15). Colocalization assays were 
analyzed by excitation of both fluorochromes in the same 
section. Negative controls, lacking the primary antibody or 
using an irrelevant antibody, showed no staining.

**Edelfosine uptake.** Drug uptake was measured as de-
scribed previously (13) after incubating $10^6$ cells with 
10 nmol $[^3]$H]edelfosine for 2 h in RPMI 1640/10% fetal 
bovine serum and subsequent exhaustive washing (six 
times) with PBS + 2% bovine serum albumin to elimi-
nate the loosely cell surface–bound ether lipid. $[^3]$H]Edel-
fosine (specific activity, 42 Ci/mmol) was synthesized by 
tritiation of the 9-octadecenyl derivative (Amersham 
Buchler).

**Cholesterol depletion.** Cells (2.5 x $10^5$/mL) were pre-
treated with 2.5 mg/mL methyl-$\beta$-cyclodextrin (MCD) 
for 30 min at 37°C in serum-free medium. Cells were then 
flushed thrice and resuspended in complete culture medi-
um before edelfosine addition.

**Xenograft mouse model.** CB17–severe combined immu-
nodeficient (SCID) mice (Charles River Laboratories), kept 
and handled according to institutional guidelines, com-
plying with Spanish legislation under a 12/12-h light/dark 
cycle at a temperature of 22°C, received a standard diet.

**Fig. 1.** Induction of apoptosis in MCL and CLL cells by ALPs. MCL (JVM-2 and Z-138) and CLL (EHEB) cell lines were incubated for the indicated times 
with 10 μmol/L of the distinct ALPs edelfosine, perifosine, miltefosine, and erucylphosphocholine (Erucyl-PC; A) or for 24 h with different concentrations 
of the ALPs (B). Apoptosis was then quantitated as percentage of cells in the sub-G1 region by flow cytometry. Untreated control cells were run in 
parallel. Data are means ± SE of four independent determinations. C, cells were untreated or treated with 10 μmol/L perifosine or edelfosine for the indicated 
times and analyzed by Western blot with anti–cyclin D1 and anti–β-actin antibodies. Immunoblotting of β-actin was used as an internal control for 
equal protein loading in each lane. Blots are representative of three experiments done.
and acidified water ad libitum. CB17-SCID mice were inoculated s.c. into their lower dorsum with 10^7 Z-138 or EHEB cells in 100 μL PBS and 100 μL Matrigel basement matrix membrane matrix (Becton Dickinson). When tumors were palpable, mice were randomly assigned into cohorts of 8 to 10 mice each, receiving a daily oral administration of edelfosine (30 mg/kg) or an equal volume of vehicle (PBS). The shortest and longest diameter of the tumor were measured with calipers at 2- to 5-d intervals, and tumor volume (mm^3) was calculated using the following standard formula: (the shortest diameter)^2 × (the longest diameter) × 0.5. Animals were sacrificed, according to institutional guidelines, when the diameter of their tumors reached 3 cm or when significant toxicity was observed. Animal body weight and any sign of morbidity were monitored. Drug treatment lasted for 21 d (MCL) and 34 d (CLL), and mice were killed 24 h after the last drug administration. Then, tumor xenografts were excised, measured, and weighed, and a necropsy analysis involving tumors and distinct organs was carried out.

**Plasma/tissue extraction procedure for edelfosine biodistribution studies.** MCL- or CLL-bearing SCID mice were treated with a daily oral administration of edelfosine (30 mg/kg) for 21 d (MCL) or 34 d (CLL). Twenty-four hours after the last drug oral administration, blood was collected in EDTA surface-coated tubes and then centrifuged at 2,000 × g for 15 min (4°C) to collect plasma (100 μL). Then, animals were sacrificed, and distinct organs and tumors were collected and weighed. Tissues and tumors were homogenized in 1-mL PBS (pH 7.4) using a Mini-bead Beater (BioSpect Products, Inc.) and centrifuged at 10,000 × g for 10 min. Both plasma and tissue supernatants were collected and stored at −80°C until high-performance liquid chromatography–mass spectrometry (HPLC-MS) analysis was done. Ten micrograms of platelet-activating factor (1 mg/L), used as internal standard, were added onto 100 μL of plasma or tissue/tumor supernatant. A mixture (190 μL) of 1% formic acid/methanol was added to precipitate proteins. Samples were vortexed for 1 min, and after centrifugation (20,000 × g, 10 min), 25 μL of the supernatant were analyzed by HPLC-MS.

**Quantitative determination of edelfosine by HPLC-MS analysis.** The technique used was a slight modification of a previously described method (27) and is described in detail in Supplementary Data.

**Statistical analysis.** All values are expressed as means ± SE. Between-group statistical differences were assessed using the Mann-Whitney test or the Student’s t test. A P value of <0.05 was considered statistically significant.

## Results

**Edelfosine is the most potent ALP inducing apoptosis in MCL and CLL cells.** We have previously found that the ALP edelfosine is effective in inducing apoptosis in several leukemic cells through the aggregation of Fas/CD95 in clustered rafts (14–18). Following time course (Fig. 1A) and dose-response (Fig. 1B) experiments, we found that ALPs ranked edelfosine > perifosine > erucylphosphocholine ≫ miltefosine for their capacity to promote apoptosis, assessed as the percentage of cells in the sub-G1 region (28), in MCL (JVM-2 and Z-138) and EHEB cell lines. Because MCL is characterized by cyclin D1 overexpression, we analyzed cyclin D1 levels by Western blot in Z-138 and JVM-2 cells exposed for 1 to 24 hours to 10 μmol/L.

**Fig. 2.** Edelfosine and perifosine kill patient-derived MCL and CLL cells. Primary malignant cells isolated from MCL and CLL patients were incubated for 48 h in the absence (control) or in the presence of 10 μmol/L edelfosine (EDLF) or perifosine (PERF), and then cell viability was assessed as nonapoptotic cells in Annexin V analysis by flow cytometry. Data are means ± SE of 7 (MCL) or 10 (CLL) independent patients. Asterisks indicate that cell viability in edelfosine- and perifosine-treated cells is significantly different from untreated control cells at P < 0.05 (*) and P < 0.01 (**) levels by Student’s t test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Cell viability</th>
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<tbody>
<tr>
<td></td>
<td>CD19+ CLL cells</td>
</tr>
<tr>
<td>Edelfosine (10 μmol/L)</td>
<td>60.3 ± 5.6</td>
</tr>
<tr>
<td>Edelfosine (20 μmol/L)</td>
<td>43.7 ± 4.9</td>
</tr>
<tr>
<td>Perifosine (10 μmol/L)</td>
<td>73.1 ± 4.8</td>
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<tr>
<td>Perifosine (20 μmol/L)</td>
<td>61.0 ± 3.7</td>
</tr>
<tr>
<td>Stauroporine (0.5 μmol/L)</td>
<td>17.8 ± 6.7</td>
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**Table 1.** Selective killing of patient-derived CLL cells by edelfosine, sparing normal cells

NOTE: Primary lymphocyte cultures from CLL patients were incubated with edelfosine or perifosine for 48 h at the indicated concentrations. Stauroporine was used as a positive control of apoptosis. Percentage of cell viability was measured as nonapoptotic CD3+/CD19− T cells or CD3−/CD19+ B cells from CLL samples in Annexin V analysis by flow cytometry. Untreated control CD19− and CD3+ cells were run in parallel and showed a cell viability of >93% and >98%, respectively. Data are shown as mean values ± SE of five independent CLL patients.
perifosine or edelfosine. As shown in Fig. 1C, we found only a decrease in cyclin D1 protein level in Z-138 cells after 24-hour treatment. However, we detected the cleavage of the typical caspase-3 substrate poly(ADP-ribose) polymerase, as a marker for apoptosis onset, by 6-hour treatment in edelfosine- and perifosine-treated Z-138 and JVM-2 cells (data not shown), whereas cyclin D1 protein level remained unaffected (Fig. 1C). It is noteworthy that despite the presence of t(11;14) in both JVM-2 and Z-138 cell lines, JVM-2 cells harbored a reduced basal cyclin D1 content when compared with Z-138, as assessed by protein and mRNA levels (Fig. 1C; data not shown). Thus, these results suggest that cyclin D1 modulation is not involved in edelfosine/perifosine antitumor activity in MCL cell lines and that the decrease in cyclin D1 protein level detected at 24 hours (Fig. 1C) was a consequence rather than a cause in edelfosine- and perifosine-induced apoptosis.

Edelfosine was also more active than perifosine against primary malignant cells derived from MCL and CLL patients (Fig. 2). Following a dose-response analysis, we found that edelfosine was significantly more cytotoxic ($P < 0.05$) than perifosine against primary CD19$^+$ CLL cells derived from patients, whereas normal resting CD3$^+$ T lymphocytes from the same patients remained rather resistant (Table 1). This selective action of both edelfosine and perifosine was highlighted when compared with staurosporine that induced apoptosis in both malignant and normal cells at a similar rate (Table 1).

Edelfosine-induced apoptosis in MCL and CLL cells is mediated by lipid rafts. By using the raft marker FITC-labeled cholera toxin B subunit that binds ganglioside GMA (29), mainly found in rafts (30), we found that 10 $\mu$mol/L edelfosine induced coclustering of lipid rafts and Fas/CD95 in both JVM-2 and EHEB cells (Fig. 3). Furthermore, we found that MCL (JVM-2 and Z-138) and CLL (EHEB) cell lines took up high amounts of edelfosine (Fig. 4A), whereas normal resting lymphocytes incorporated negligible amounts of drug (<20 pmol/10$^6$ cells after
2 h of incubation with 10 nmol [3H]edelfosine). Lipid raft disruption by MCD treatment inhibited drug uptake (Fig. 4A), edelfosine-induced apoptosis (Fig. 4B), as well as Fas/CD95 clustering (data not shown). These data suggest that lipid rafts are essential for edelfosine uptake and edelfosine-induced apoptosis in MCL and CLL cells.

Edelfosine accumulates in MCL and CLL tumors and inhibits human MCL and CLL cell growth in vivo. We next determined whether edelfosine showed in vivo anti-human MCL and CLL cell activity. CB17-SCID mice were inoculated with 10^7 Z-138 or EHEB cells for MCL or CLL xenograft animal models. The MCL animal model developed aggressive tumors that were tangible within 1 week of inoculation, and increased rapidly in a few weeks time (Fig. 5A), whereas CLL tumors grew more slowly (Fig. 5A). Following the development of a palpable tumor, mice were randomized into drug-treated (30 mg/kg edelfosine, daily oral administration) and control (PBS vehicle) groups. Caliper measurements were done every 2 to 5 days to calculate tumor volume (Fig. 5A). Oral administration of edelfosine achieved significant MCL and CLL tumor regression (Fig. 5A-C). When tumor size reached ~10% of body weight, animals were killed for ethical reasons. This size was approximately reached by day 15 in some of the MCL tumor-bearing mice (Fig. 5A), but this animal model was extended 1 week more to obtain reliable and comparable data with drug-treated animals in which tumor size was decreased by that time (Fig. 5A). A comparison of tumors isolated from untreated control and drug-treated MCL- and CLL-bearing mice, at the end of treatment, rendered a remarkable anti-MCL and anti-CLL activity of edelfosine, with a reduction of >85% in tumor weight and volume in both MCL and CLL animal models (Fig. 5B). Organ examination at necropsy did not reveal any apparent toxicity (data not shown), and there was an evident difference between the highly vascularized tumors from drug-free mice and the pale poorly vascularized tumors from edelfosine-treated mice (Fig. 5C). In addition, MCL tumors were bulky in drug-free mice but resulted rather flat after edelfosine treatment (Fig. 5C). No significant differences in mean body weight were observed between drug-treated and control animals during the in vivo assay (3-5% of body weight loss in the treated groups versus control groups). A drug biodistribution study showed that edelfosine dramatically accumulated in the MCL and CLL tumors (Fig. 5D). Tumor/plasma concentration ratio of edelfosine in the tumor was significantly higher than that detected in both kidney and liver after completion of the experiment in MCL and CLL animal models (Fig. 5D), with a drug mean concentration in plasma of 5.64 μg/mL. In the CLL animal model, we examined the content of edelfosine in a wide variety of distinct organs and found that the drug was dramatically accumulated in the tumor compared with lung, heart, spleen, liver, intestine, or kidney (Fig. 5D). Taken together, our data indicate a preferential accumulation of edelfosine in the tumor.

Discussion

The data reported here show that edelfosine behaves as the most potent ALP in killing MCL and CLL cells via a raft-mediated process. Our data indicate that edelfosine is a powerful antitumor agent against MCL and CLL as assed by in vitro, ex vivo, and in vivo evidences. In addition, we found a rather selective and dramatic accumulation of edelfosine in MCL and CLL tumor cells in animal models. Here, we found that edelfosine induces the recruitment of Fas/CD95 death receptor in raft aggregates in MCL and CLL cells. Raft disruption by cholesterol depletion in MCL and CLL cells inhibited both edelfosine uptake and drug-induced apoptosis, as well as Fas/CD95 clustering, thus suggesting a major role of rafts in the uptake and antitumor action of edelfosine. Previous reports have shown that MCL and CLL cells express Fas/CD95, but a deficient apoptotic response to the external stimulation of Fas/CD95 by agonistic anti-Fas/CD95 antibodies was reported (7, 31). Unlike the natural ligand FasL/CD95L or agonistic anti-Fas/CD95 antibodies that act through their interaction with

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the exogenous portion of the Fas/CD95 death receptor, edelfosine induces activation of Fas/CD95 from within the cell independently of its ligand (16, 32). We have previously found that edelfosine is even more efficient than FasL/CD95L in promoting programmed cell death through Fas/CD95 activation by its recruitment in membrane rafts enriched in downstream signaling molecules (14, 16–18, 25, 33). Thus, edelfosine might induce Fas/CD95 activation, although the receptor is not triggered by its natural ligand or agonistic antibodies.

Using distinct MCL and CLL xenograft mouse models, we found that edelfosine accumulates in high amounts in the tumor tissue and shows a remarkable antitumor activity, leading to dramatic tumor regression. In addition, we consistently found in the MCL and CLL xenograft animal models that tumors became smaller and poorly vascularized. This could be in agreement with reports showing an antiangiogenic effect of edelfosine (34, 35). Thus, further insight into the effect of edelfosine on angiogenesis and how this action affects cancer development is warranted.

Following edelfosine oral administration in non–tumor-bearing SCID mice, we have recently found a rather wide drug distribution pattern to several tissues, including lung, spleen, intestine, liver, and kidney (36). In this study, we also found that edelfosine showed a preferential accumulation in the tumor in a MCL-bearing mouse animal model (36). Now, we have largely extended this initial study and analyzed the in vivo effect of edelfosine in MCL and CLL animal models. Interestingly, we found here that when

**Fig. 5.** Edelfosine inhibits human MCL and CLL cell growth in vivo. CB17-SCID mice were inoculated s.c. with 10^7 Z-138 or EHEB cells. Daily oral administration of edelfosine (30 mg/kg, n = 10 for MCL animal model and n = 8 for CLL animal model) started after the development of a palpable tumor. Tumor size was recorded every 2 to 5 d. A, edelfosine significantly (P < 0.01; from day 15 of treatment until the end of the experiment) inhibited MCL and CLL tumor growth compared with the control group treated with vehicle (PBS, n = 10 for MCL animal model and n = 8 for CLL animal model). Data are means ± SE (n = 10, MCL; n = 8, CLL). B, after completion of the in vivo assay (22 d for MCL and 35 d for CLL), control and edelfosine-treated mice were sacrificed and tumors were measured in the distinct mice. The tumor size and weight values of each single animal (dots) and the average values of each experimental group (horizontal bars) are shown. Asterisks indicate that tumor weight and size values were significantly lower after edelfosine treatment, compared with control drug-free mice, at P < 0.01 (**). C, remarkable growth inhibition of MCL and CLL tumors was observed after edelfosine treatment (30 mg/kg). D, tissue/plasma concentration ratios, in liver, kidney, and tumor, of edelfosine after daily oral administration of edelfosine (30 mg/kg) for 3 wk in MCL-bearing SCID mice (mean ± SE, n = 5), and tissue/plasma concentration ratios, in lung, heart, spleen, liver, intestine, kidney, and tumor, of edelfosine after daily oral administration of edelfosine (30 mg/kg) for 34 d in CLL-bearing SCID mice (mean ± SE, n = 5). Asterisks indicate that the tumor/plasma ratio values are significantly different from the other tissue/plasma ratios at P < 0.01 (**).
SCID mice contained a MCL or CLL tumor, edelfosine distribution was dramatically and significantly shifted toward the tumor (tissue/plasma concentration ratios >16; P < 0.01), suggesting a preferential tumor location for edelfosine. Our herein reported in vivo data, together with our present and previous in vitro determinations in a wide number of malignant and normal cells (14, 16, 17), suggest a rather selective edelfosine uptake and cytotoxic action in tumor cells. The selective action of edelfosine on tumor cells supports its low toxicity. We did not find any apparent damage in the distinct organs analyzed following necropsy analysis in the in vivo studies reported here. Lack of toxicity of edelfosine in a rat model has been reported, including no significant cardiotoxicity, hepatotoxicity, or renal toxicity (37). Our biodistribution data in the murine models reported here showed a mean concentration of edelfosine in plasma of 5.64 μg/mL (10.77 μmol/L; edelfosine molecular mass, 523.7). Thus, the herein reported in vitro effects, rendered by 10 μmol/L edelfosine, were detected at a pharmacologically relevant drug concentration.

Our data constitute the first in vitro and in vivo evidence for the antitumor action of edelfosine in MCL and CLL, two hematologic malignancies with poor survival outcome. Taken together, the results reported here provide the proof of principle and rationale for further clinical evaluation of edelfosine to improve patient outcome in MCL and CLL. The results reported here also highlight the involvement of lipid rafts in the action of edelfosine on B-cell malignancies, such as MCL and CLL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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