

Aloe-emodin Is a New Type of Anticancer Agent with Selective Activity against Neuroectodermal Tumors¹

Teresa Pecere, M. Vittoria Gazzola, Carla Mucignat, Cristina Parolin, Francesca Dalla Vecchia, Andrea Cavaggioni, Giuseppe Basso, Alberto Diaspro, Benedetto Salvato, Modesto Carli, and Giorgio Palù²

Department of Histology, Microbiology, and Medical Biotechnologies, Medical School [T. P., C. P., G. P.], Division of Oncology and Hematology, Department of Pediatrics, Medical School [M. V. G., G. B., M. C.], Department of Human Anatomy and Physiology, Medical School [C. M., A. C.], and Department of Biology [F. D. V., B. S.], University of Padova, 35100 Padova and Istituto Nazionale Fisica della Materia and Department of Physics, University of Genova, 16146 Genova [A. D.], Italy

Abstract

Here we report that aloe-emodin (AE), a hydroxyanthraquinone present in *Aloe vera* leaves, has a specific *in vitro* and *in vivo* antineuroectodermal tumor activity. The growth of human neuroectodermal tumors is inhibited in mice with severe combined immunodeficiency without any appreciable toxic effects on the animals. The compound does not inhibit the proliferation of normal fibroblasts nor that of hemopoietic progenitor cells. The cytotoxicity mechanism consists of the induction of apoptosis, whereas the selectivity against neuroectodermal tumor cells is founded on a specific energy-dependent pathway of drug incorporation. Taking into account its unique cytotoxicity profile and mode of action, AE might represent a conceptually new lead antitumor drug.

Introduction

With the aim of developing novel anticancer drugs characterized by selective targeting and low toxicity for dividing normal host tissues, we devoted our attention to a number of natural compounds that have traditionally been used to treat a variety of diseases for hundreds of years (1–3). We assayed only those natural compounds that have already been proven to be nontoxic, and we evaluated their efficacy against highly malignant tumors that are not normally included in the classical screening assays, *i.e.*, pPNET,³ Ewing's sarcoma, and neuroblastoma. The last of these is the most common solid extracranial tumor in infants, accounting for 10% of all childhood cancers. At the time of diagnosis, ~50% of affected children have disseminated neuroblastoma disease with a very poor prognosis that has remained unchanged in the last 3 decades (4, 5). Our study analyzed the cytotoxic potential of AE, a hydroxyanthraquinone (Fig. 1A) naturally present in the leaves of *Aloe vera* (6, 7). This report describes the selective *in vitro* and *in vivo* killing of neuroectodermal tumor cells by AE, the anticancer activity of which is based on apoptotic cell death, promoted by a tumor cell-specific drug uptake process that may offer opportunities for novel anticancer agents.

Materials and Methods

Drugs. AE was purchased from Sigma-Aldrich (Milan, Italy); it was dissolved in DMSO to reach a concentration of 200 mM and stored at –20°C. The

compound was diluted in the appropriate medium immediately before use. AE is a fluorescent compound with a maximum excitation wavelength at 410 nm and a maximum emission wavelength at 510 nm. Aloin was a generous gift of MacFarlan Smith Ltd. (Edinburgh, Scotland). It was dissolved by slight warming in saline solution at the working concentration.

Mice. Female SCID mice were purchased from Charles River Italia (Calco, Italy). The animals were kept in a pathogen-free colony, in microisolators, and were fed sterile pellets and sterile water *ad libitum*. During the experiments, mice were tested for the presence of gross sensory or motor neurological disturbances: the geotaxic response and righting reflexes, forelimb placement reflex, and climbing responses (8). Hematological assessment was performed by Coulter MAXM. The animals were scored twice a week for body weight and daily for fecal emission. The mice were age matched (6–8 weeks of age) at the beginning of each experiment.

Cell Culture. Neuroblastoma cells (IMR-32, IMR-5, AF8, and SJ-N-KP), pPNET cells (TC32), Ewing's sarcoma cells (TC106), T-cell leukemia cells (CEM), and vinblastine-resistant cells (CEM VBL), colon adenocarcinoma cells (LoVo 109), and doxorubicin-resistant cells (LoVo DX) were cultured in RPMI 1640 supplemented with 25 mM HEPES buffer and with 2 mM L-glutamine (all from Life Technologies, Ltd., Paisley, Scotland). The culture of CEM VBL cells was supplemented with 10 µg/ml vinblastine (Lilly France, Saint-Cloud, Paris, France), and the culture of LoVo DX cells was supplemented with 0.1 µg/ml doxorubicin (Pharmacia, Milan, Italy). Cervix epithelioid carcinoma (HeLa) and human lung fibroblast (MRC5) cells were cultured in DMEM supplemented with 25 mM HEPES buffer and with 2 mM L-glutamine (all from Life Technologies, Ltd.). All culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, Milan, Italy), 100 units/ml penicillin, and 100 µg/ml streptomycin (Sigma-Aldrich, Irvine, United Kingdom). All cell lines were grown at 37°C with 5% CO₂ humidified atmosphere.

In Vitro Cytotoxicity. The cytotoxic activity of AE was determined in exponentially growing cells in complete medium over 72 h. The cells were seeded in 12 wells/plate 24 h before the treatment; monolayer cells were plated at a density of $5\text{--}7 \times 10^4$ cells/well, and suspension cells were plated at 40×10^4 cells/well. AE was added to the experimental final concentration, and cells were counted 72 h later using the trypan blue exclusion assay. All of the experiments were conducted at least in triplicate.

Hemopoietic Progenitors and Neuroblastoma Colony Assay. MNCs from BM aspirates and CB samples and from neuroblastoma cell lines (SJ-N-KP and AF8) were cultured in methylcellulose medium supplemented with a combination of recombinant colony-stimulating factors (Stem Cell Technologies, Vancouver, British Columbia, Canada). Cells were plated in triplicate at the concentration of 5×10^4 /ml for BM- and CB-MNC, and 1×10^3 for NB cells, in 35-mm-diameter dishes (Becton Dickinson, Franklin Lakes, NJ) and incubated at 37°C in a 5% CO₂ humidified atmosphere. MNC and NB cell lines were cultured in the absence or in the presence of different concentrations of AE. On day 14 of culture, the number of CFU-GM and neuroblastoma colonies was counted with an inverted microscope (Leitz-Diavert). All of the experiments were conducted at least three times.

Fluorescence-activated Cell Sorting Analysis. Neuroblastoma (SJ-N-KP), colon adenocarcinoma (LoVo 109), and cervix epithelioid carcinoma (HeLa) cell lines (1×10^6) were cultured for different time periods in the presence of AE or drug-free medium. Cells were harvested, washed twice with PBS, and fixed with cold 70% ethanol at 4°C. After centrifugation of the

Received 3/16/00; accepted 4/19/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by a grant from Associazione Italiana lotta alla Leucemia (Sezione di Padova; to T. P.), by grants from Regione Veneto, Fondazione Cassa di Risparmio di Padova e Rovigo, ISS-AIDS, CNR-Biotechnology (to G. P.) and financial support from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST).

² To whom requests for reprints should be addressed, at Department of Histology, Microbiology, and Medical Biotechnologies, Medical School, University of Padova, via Gabelli 63, 35121 Padova, Italy.

³ The abbreviations used are: pPNET, primitive peripheral neuroectodermal tumor; AE, aloe-emodin; SCID, severe combined immunodeficiency; MNC, mononuclear cell; BM, bone marrow; CB, cord blood; CFU-GM, colony forming unit-granulocyte/macrophage; TPE, two-photon excitation; TEM, transmission electron microscopy.

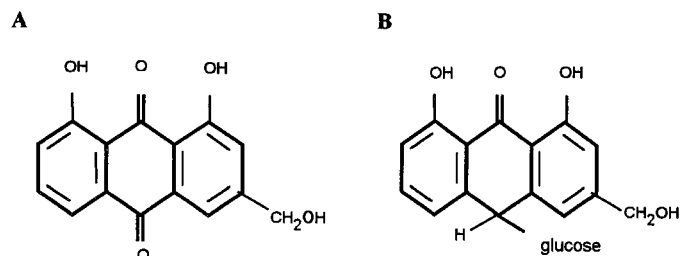


Fig. 1. Chemical structure of AE (1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione; A) and aloin (10-(10'-1',5'-anhydroglucosyl)-aloe-emodin-9-anthrone; B).

samples, propidium iodide (50 $\mu\text{g}/\text{ml}$ in PBS) and RNase were added to the pellet for 20 min at 37°C to determinate the effect of AE on the cell cycle dynamics. DNA fluorescence was measured by flow cytometry (EPICS XL; Coulter, Miami, FL) analysis according to a published method (9). To determinate drug uptake, SJ-N-KP, LoVo 109, and HeLa cells were cultured in the presence of 25 μM of AE or in drug-free medium at 37°C or at 4°C or in presence of NaN_3 , for 24 h and then analyzed by flow cytometry (10).

Two-Photon Excitation Microscopy. Neuroblastoma (IMR5), colon adenocarcinoma (LoVo 109), and cervix epithelioid carcinoma (HeLa) cell lines were seeded on microscope coverslips in 12-well plates and cultured with drug-free medium 24 h before treatment. Then AE was added at different concentrations. At different time points, cells were washed twice with PBS and examined by means of "fluorescence two-photon confocal microscopy." Optical sections were acquired with a TPE architecture described in detail elsewhere (11).

Transmission Electron Microscopy Analysis. Cells were cultured with different concentrations of AE or with drug-free medium. At 24 and 48 h cells were scraped, washed twice in PBS, and fixed overnight at 4°C in 3%

glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.9) and then processed according to Ciman *et al.* (12). Ultrathin sections, cut with an ultramicrotome (Ultracut; Reichert-jung), were observed with the transmission electron microscope (TEM 300; Hitachi) operating at 75 kV.

Results

Cytotoxic Activity of AE in Cell Culture. The cytotoxic potential of AE was evaluated on exponentially growing cells over a period of 72 h. As shown in Fig. 2A, AE displayed a specific dose-dependent cytotoxic effect on neuroblastoma, pNET, and Ewing's sarcoma cells. Indeed, the growth of the neuroectodermal tumor cell lines was specifically inhibited, and ED_{50} s (half-maximal effective doses) ranged between 1 and 13 μM (neuroblastoma and Ewing's sarcoma, respectively). Conversely, epithelial tumors, such as cervix carcinoma and colon carcinoma cells, and also T-cell leukemia cells and normal fibroblasts, were almost refractory to the treatment with AE (Fig. 2B). ED_{50} s for these cell lines ranged from 40 μM for cervix carcinoma cells (HeLa) to 100 μM for T-cell leukemia cells (CEM).

To determine whether AE might have inhibited the clonogenic activity of the hemopoietic progenitors and neuroectodermal tumor cell lines, cells were seeded into methylcellulose medium and monitored for colony formation. As shown in Fig. 2C, AE had no significant inhibitory activity on the growth of CFU-GM after 14 days of treatment. The colony growth was only partially reduced at high concentrations of AE, with ED_{50} s of 80 and 120 μM , respectively, for BM- and CB-derived CFU-GMs. In contrast, the colony-forming activity of neuroblastoma cells (SJ-N-KP) was inhibited at a much lower concentration of AE (ED_{50} of 7 μM).

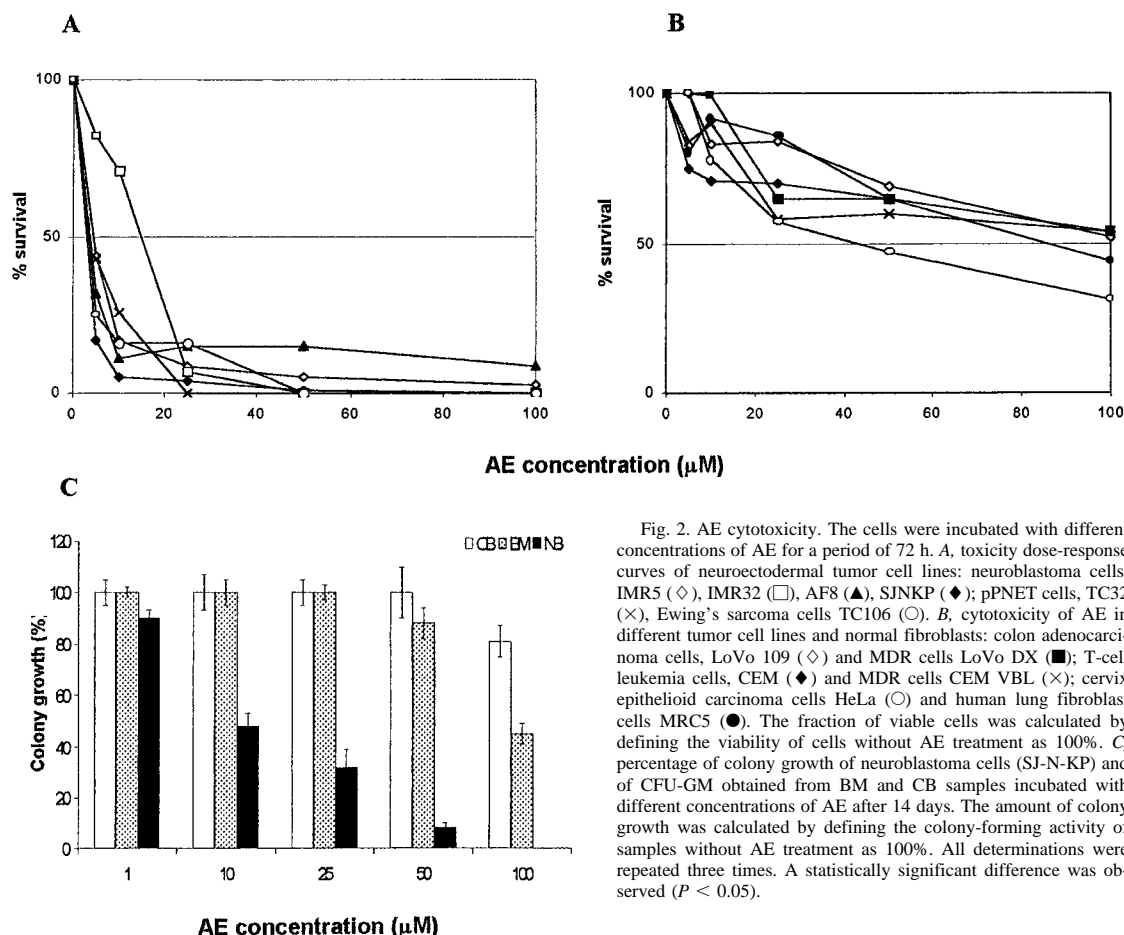


Fig. 2. AE cytotoxicity. The cells were incubated with different concentrations of AE for a period of 72 h. A, toxicity dose-response curves of neuroectodermal tumor cell lines: neuroblastoma cells, IMR5 (\diamond), IMR32 (\square), AF8 (\blacktriangle), SJNKP (\blacklozenge); pNET cells, TC32 (\times), Ewing's sarcoma cells TC106 (\circ). B, cytotoxicity of AE in different tumor cell lines and normal fibroblasts: colon adenocarcinoma cells, LoVo 109 (\diamond) and MDR cells LoVo DX (\blacksquare); T-cell leukemia cells, CEM (\blacklozenge) and MDR cells CEM VBL (\times); cervix epithelioid carcinoma cells HeLa (\circ) and human lung fibroblast cells MRC5 (\bullet). The fraction of viable cells was calculated by defining the viability of cells without AE treatment as 100%. C, percentage of colony growth of neuroblastoma cells (SJ-N-KP) and of CFU-GM obtained from BM and CB samples incubated with different concentrations of AE after 14 days. The amount of colony growth was calculated by defining the colony-forming activity of samples without AE treatment as 100%. All determinations were repeated three times. A statistically significant difference was observed ($P < 0.05$).

Specific Uptake of AE by Neuroectodermal Tumor Cell Lines and Intracellular Localization. To explain the specific cytotoxic activity of AE against neuroectodermal tumor cell lines, we evaluated the cellular uptake of this compound by different cell lines, exploiting the drug's relatively intense green fluorescence (see "Materials and Methods"). As shown in Fig. 3, A and B, AE treatment of SJ-N-KP and HeLa cells at 37°C gave rise to an intense fluorescence emission only from the former, a result suggestive of AE selective cellular uptake. Conversely, when SJ-N-KP cells were exposed to AE at 4°C, no fluorescence emission was detected (Fig. 3C). With colorectal carcinoma (LoVo 109) and T-cell leukemia (CEM) cell lines, lack of drug uptake was also observed at 37°C (data not shown).

To determine whether drug accumulation depended on the energized status of the cells, ATP pools were depleted by a 2-h preincubation with 1 mM NaN_3 in glucose-free medium (13). Fig. 3D shows that this led to a significant decrease in intracellular fluorescence emission by SJ-N-KP cells.

Microscopic observation with TPE modality showed the relative amount of AE uptake and provided three-dimensional information on the drug's intracellular fate in sensitive cells. After 24 h of incubation, AE was present in the cytoplasm of neuroblastoma cell lines in a spotty fashion inside endosomes (Fig. 3E), with an intensity of fluorescence of 8.8 arbitrary units. A barely detectable fluorescence emission (1.9 arbitrary units) was recovered from HeLa (Fig. 3F), LoVo 109, and MRC5 cells maintained under the same experimental conditions.

Nuclear localization of AE was readily appreciable in the sensitive cells as early as 1 h after treatment. In this case, because of the intrinsic fluorescence quenching of the anthraquinone on interaction with DNA (14), drug detection was achieved by counterstaining nuclei with propidium iodide (data not shown).

Effects of AE on Cell Cycle and Apoptosis. On the basis of its chemosensitivity profile, neuroblastoma was selected as a prototype chemosensitive tumor for exploring the molecular requirements for AE-triggered cell death. Changes in SJ-N-KP cellular proliferation (DNA content and distribution) during treatment with AE were monitored by flow cytometry over a period of 48 h, an interval sufficient for SJ-N-KP cells to complete a cell cycle. As shown in Fig. 4, A and B, after 24 h of treatment a relevant proportion of the cells remained in the G_2 -M phase of the cycle (20%). After 48 h, a sub- G_0 peak (60%) was observed, suggestive of the presence of apoptotic cells with fragmented DNA (Fig. 4C). Typical morphological features of apoptotic cell death, with cell shrinkage, membrane blebbing, and nuclear fragmentation, were also exhibited by most AE-treated cells at TEM analysis. A representative picture of this phenomenon is shown in Fig. 4, D and E.

In Vivo Inhibition of Neuroblastoma Growth by AE. The potential of AE as an antineoplastic agent *in vivo* was assessed in a murine model system. Mice with SCID were injected s.c. with human neuroblastoma cells (IMR5) and immediately treated with AE at a dose of 50 mg/Kg/day (the highest concentration compatible with an aqueous solution of the drug). The tumor was sensitive to the drug, as shown by a significant reduction ($P < 0.05$) of its growth in the animal hosts (Fig. 5A). Furthermore, when AE treatment was delayed until a palpable tumor mass had developed (day 15), tumor growth was halted (Fig. 5B) throughout the period of drug administration ($P < 0.05$). As seen *in vitro* (Fig. 2B), the human colon carcinoma cell line (LoVo 109) injected s.c. into SCID mice was refractory to the treatment (Fig. 5C). No appreciable signs of acute or chronic toxicity were observed in any of the treated animals; weight, neurological and intestinal functions, and hematological parameters were normal, and no other manifestation of acute toxicity was evident. No structural

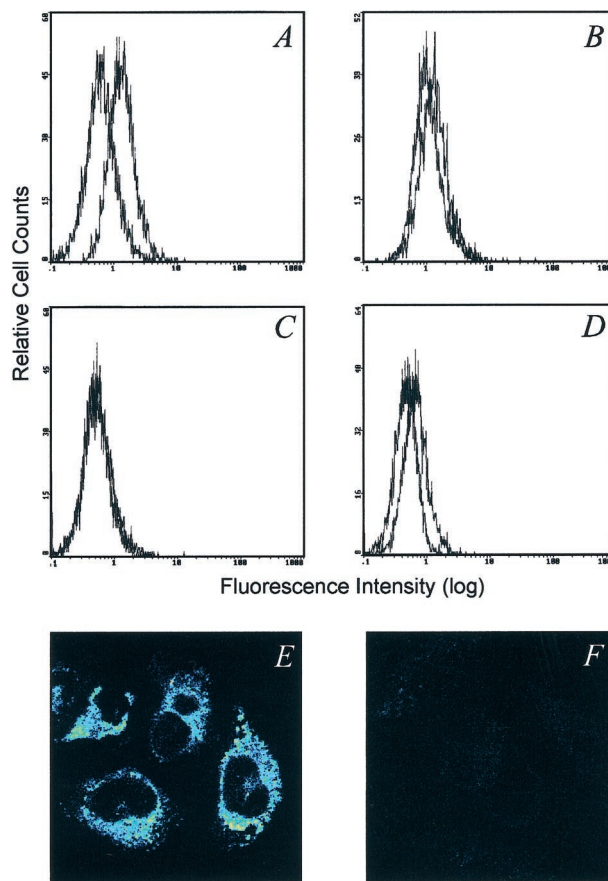


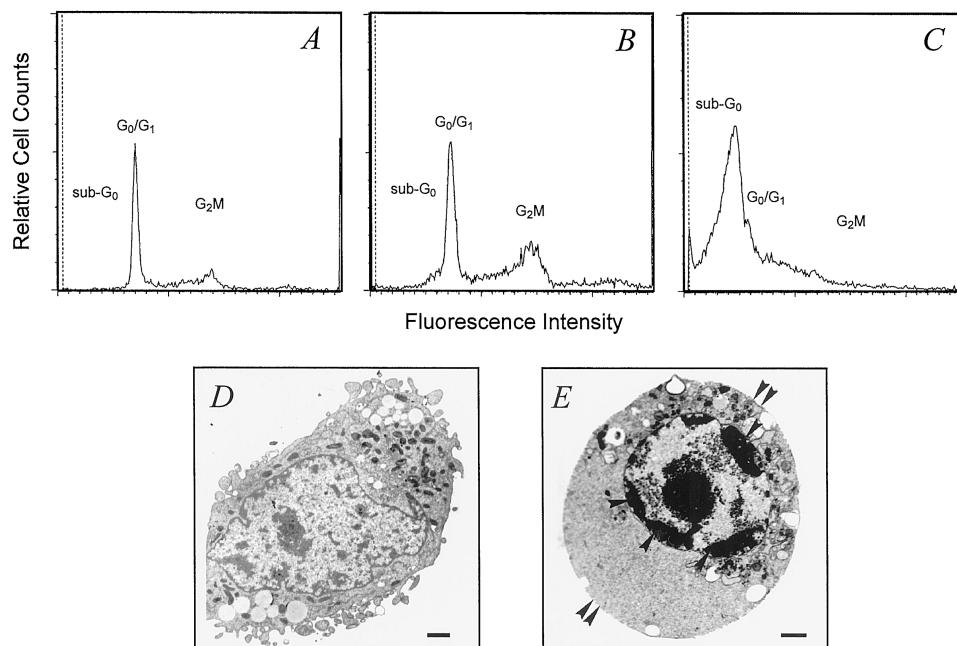
Fig. 3. Flow cytometry analysis of AE cellular uptake. All cell cultures were incubated with 25 μM of AE for 24 h. Neuroblastoma (SJ-N-KP; A) and cervix carcinoma (HeLa; B) cells were incubated at 37°C. C, SJ-N-KP cells were incubated at 4°C. D, SJ-N-KP cells with depleted ATP pools were treated for 24 h at 37°C. The cellular uptake of AE was measured by flow cytometry analysis using AE green fluorescence (right line). Untreated cells were used for control purpose (left line). Intracellular drug distribution analysis by TPE microscopy in neuroblastoma cells (E) and in cervix carcinoma cells (F) treated for 24 h with 25 μM of AE.

abnormalities were observed on macroscopic examination in either the AE-treated or control group.

Discussion

Here we show that AE, a hydroxyanthraquinone present in *Aloe vera* leaves, selectively inhibits human neuroectodermal tumor cell growth in tissue cultures and in animal models. Neuroblastoma, pPNET, and Ewing's sarcoma cells were found highly susceptible to AE, whereas human malignant cells from epithelial and blood-derived tumors, as well as human hemopoietic progenitors and normal fibroblasts, were not sensitive to this compound. This is the first report that describes the potential antitumor activity of AE. Other groups had already investigated AE as a cytotoxic agent on several tumor cell lines, but no significant activity was found. In this regard, Driscoll *et al.* (15) assayed 379 anthraquinone derivatives against L-1210 leukemia in mice and included AE among the inactive compounds. Grimaudo *et al.* (16) reported that AE was endowed with some degree of cytotoxicity for erythroleukemia cell lines but only at high concentrations. On the other hand, Schörkhuber *et al.* (17) demonstrated a stimulatory effect of AE on urokinase secretion and colorectal carcinoma cell growth. Here we show that AE is selectively toxic against neuroectodermal tumors and inhibits human neuroectodermal tumor growth in an animal model with no evidence of acute or chronic toxicity. Lack of toxicity in combination with significant antitumor

Fig. 4. Effect of AE on cell cycle dynamics determined by flow cytometry. A, DNA fluorescence flow cytometric profiles of propidium iodide-stained neuroblastoma cells before AE treatment; DNA fluorescence flow cytometric profiles of propidium iodide-stained neuroblastoma cells after 24 (B) and 48 (C) h of incubation with AE. TEM analysis: D, neuroblastoma cell line in standard culture; E, neuroblastoma cells treated with AE for 48 h. Note the capping of chromatin (single-head arrows) and the loss of cell surface membrane protrusions (double-head arrows). Bars, 1 μ m.



activity results in a favorable therapeutic index. Our study describes the discovery of AE as a new type of anticancer agent possessing an unprecedented cytotoxic mechanism. A specific intracellular uptake was responsible for the selective toxicity of AE against human neuroectodermal tumor cells. Measurements performed at 37°C showed a

high level of incorporation of the compound into the tumor cells of neuroectodermal origin but not into other tumor cells. When neuroblastoma cells were exposed to the drug at 4°C, however, AE uptake was completely abolished. A similar result was obtained when the cells were ATP depleted, indicating that drug influx was an energy-

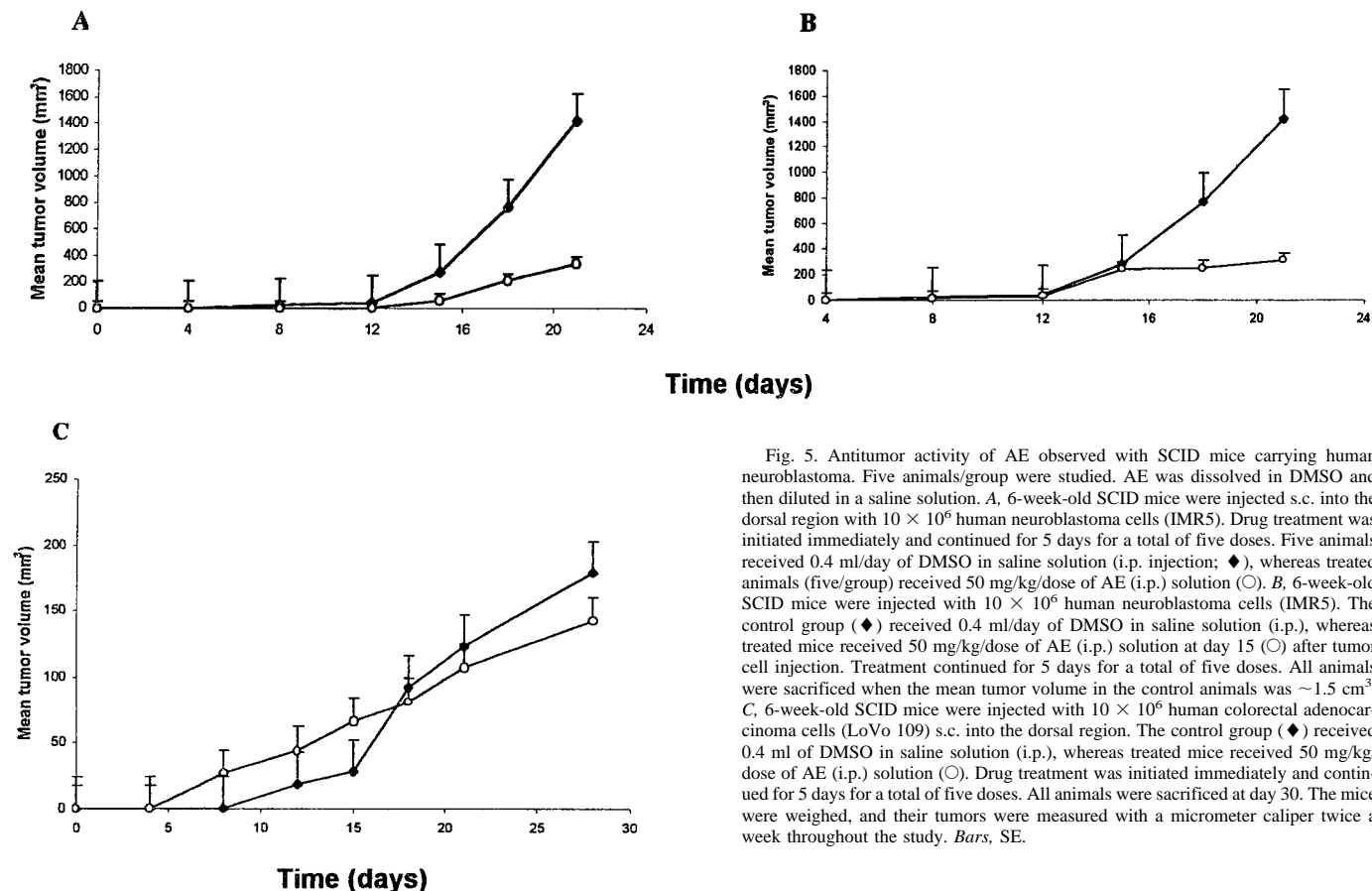


Fig. 5. Antitumor activity of AE observed with SCID mice carrying human neuroblastoma. Five animals/group were studied. AE was dissolved in DMSO and then diluted in a saline solution. A, 6-week-old SCID mice were injected s.c. into the dorsal region with 10×10^6 human neuroblastoma cells (IMR5). Drug treatment was initiated immediately and continued for 5 days for a total of five doses. Five animals received 0.4 ml/day of DMSO in saline solution (i.p. injection; \blacklozenge), whereas treated animals (five/group) received 50 mg/kg/dose of AE (i.p.) solution (\circ). B, 6-week-old SCID mice were injected with 10×10^6 human neuroblastoma cells (IMR5). The control group (\blacklozenge) received 0.4 ml/day of DMSO in saline solution (i.p.), whereas treated mice received 50 mg/kg/dose of AE (i.p.) solution at day 15 (\circ) after tumor cell injection. Treatment continued for 5 days for a total of five doses. All animals were sacrificed when the mean tumor volume in the control animals was ~ 1.5 cm³. C, 6-week-old SCID mice were injected with 10×10^6 human colorectal adenocarcinoma cells (LoVo 109) s.c. into the dorsal region. The control group (\blacklozenge) received 0.4 ml of DMSO in saline solution (i.p.), whereas treated mice received 50 mg/kg/dose of AE (i.p.) solution (\circ). Drug treatment was initiated immediately and continued for 5 days for a total of five doses. All animals were sacrificed at day 30. The mice were weighed, and their tumors were measured with a micrometer caliper twice a week throughout the study. Bars, SE.

dependent process. The nature of this process, apparently unique to neuroectodermal tumor cells, is not related to passive diffusion, nor is it likely to depend on membrane partition phenomena, which occur only at 37°C and in fully energized cells. On the other hand, when the chromophore structure of AE was modified by the presence of a hydrophilic glycosidic residue, as in aloin, a natural glucoside whose AE is the aglycone (Fig. 1B), no incorporation occurred and no cytotoxicity was exhibited in susceptible cells (data not shown). Our data would thus point to a receptor-mediated recognition process behind selective AE uptake.

Morphological observations of AE-treated neuroectodermal tumor cells revealed the typical features of apoptosis, an effect produced by many anticancer drugs (18, 19). The apoptotic phenomenon was further confirmed by the detection of a sub-G₀ peak, at flow cytometry, after 48 h of treatment. The induction of programmed cell death might be related to induction of DNA damage, as suggested by cytosolic and nuclear localization time courses.

Because of the nonselective mechanisms of action of common anticancer drugs, a high incidence of potentially severe toxicity must be tolerated for effective doses to be administered (20). In this regard, it is noteworthy that AE does not inhibit the proliferation of hematopoietic progenitors. In fact, the colony-forming activity of CFU-GM from BM and CB is not suppressed at concentrations even a hundred times higher than those inhibiting neuroectodermal tumor cell growth and clonogenic activity. This finding is at variance with the behavior of all anticancer agents in use to date and points to a novel selective mechanism residing in specific tumor targeting by a naturally available compound.

Taking into account its unique *in vitro* and *in vivo* antitumor activity, selective toxicity, and cellular pharmacokinetics, AE can be viewed as a conceptually new lead anticancer agent that might contribute to the development of targeted nontoxic drugs. Preclinical development is clearly warranted and is currently under way to explore the potential use of AE for the primary or adjuvant treatment of human neuroblastoma.

Acknowledgments

We are grateful to Paolina Mariani for support, to Arianna Calistri, Francesca Gennari, and Rossella Marcucci for helpful comments and suggestions, to Elisa Franchin, Federico Dal Bello, and Monica Spinelli for technical support; and to Francesco Bracco and Lucia Masiero for assistance in the statistical analysis.

References

1. Cassidy, J. M., and Douros, J. D. Anticancer agents based on natural product models. New York: Academic Press, 1980.
2. Cragg, G. M. Role of plants in the National Cancer Institute Drug Discovery and Development Program. In: A. D. Kinghorn and M. F. Balandrin (eds.), *Human Medicinal Agents from Plants*, pp. 80–95. Washington, DC: American Chemical Society Books, 1993.
3. Grindlay, D., and Reynolds, T., The *Aloe vera* phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol.*, 16: 117–151, 1986.
4. Carli, M., Green, A. A., Hayes, F. A., Rivera, G., and Pratt, C. B. Therapeutic efficacy of single drugs for childhood neuroblastoma: a review. In: C. Reybaud, R. Clement, G. Lebreuil, and J. L. Bernard (eds.), *Pediatric Oncology-International Congress Series 570*, pp. 141–150. Amsterdam: Excerpta Medica, 1982.
5. Brodeur, G. M., and Castellberry, R. P. Neuroblastoma. In: P. A. Pizzo and D. G. Poplack (eds.), *Principles and Practice of Pediatric Oncology*, pp. 761–797. Philadelphia: J. B. Lippincott Co., 1997.
6. Reynolds, T. The compounds in Aloe leaf exudates: a review. *Bot. J. Linn. Soc.*, 90: 157–177, 1985.
7. Fairbairn, J. W. Natural anthraquinone drugs. *Pharmacology*, 20 (Suppl. 1): 2–122, 1980.
8. Wolf, L. W., La Regina, M. C., and Tolbert, D. L. A behavioral study of the development of hereditary cerebellar ataxia in the snaker mutant rat. *Behav. Brain Res.*, 75: 67–81, 1996.
9. Nicoletti, I., Migliorati, G., Pagliacci, M. C., Grignani, F., and Riccardi, C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J. Immunol. Methods*, 139: 271–279, 1991.
10. Ricotti, E., Fagioli, F., Garelli, E., Linari, C., Crescenzo, N., Horenstein, A. L., Pistamiglio, P., Vai, S., Berger, M., Cordero di Montezemolo, L., Madon, E., and Basso, G. *c-kit* is expressed in soft tissue sarcoma of neuroectodermic origin and its ligand prevents apoptosis of neoplastic cells. *Blood*, 91: 2397–2405, 1998.
11. Diaspro, A., Corosu, M., Ramoino, P., and Robello M. Two-photon excitation imaging based on a compact scanning head. *IEEE Eng. Med. Biol.*, 18: 18–22, 1999.
12. Ciman, M., Rascio, N., Pozza, D., and Sartorelli, L. Synaptosome-free rat brain mitochondrial preparation: an improved method. *Neurosci. Res. Commun.*, 11: 87–92, 1992.
13. Hazlehurst, L. A., Foley, N. E., Gleason-Guzman, M. C., Hacker, M. P., Cress, A. E., Greenberger, L. W., De Jong, M. C., and Dalton, W. S. Multiple mechanisms confer drug resistance to mitoxantrone in the human 8226 myeloma cell line. *Cancer Res.*, 59: 1021–1028, 1999.
14. Palù, G., Palumbo, M., Antonello, C., Meloni, G. A., and Marciani-Magno, S. A search for potential antitumor agents: biological effects and DNA binding of a series of anthraquinone derivatives. *Mol. Pharmacol.*, 29: 211–217, 1985.
15. Driscoll, J. S., Hazard, G. F., Jr., Wood, H. B., Jr., and Goldin, A. Structure-antitumor activity relationships among quinone derivatives. *Cancer Chemother. Rep.*, 2, 4: 1–362, 1974.
16. Grimaudo, S., Tolomeo, M., Gancitano, R. A., D'Alessandro, N., and Aiello, E. Effects of highly purified anthraquinoid compounds from *Aloe vera* on sensitive and multidrug resistant leukemia cells. *Oncol. Rep.*, 4: 341–343, 1997.
17. Schörkhuber, M., Richter, M., Dutter, A., Sontag, G., and Marian, B. Effect of anthraquinone-laxatives on the proliferation and urokinase secretion of normal pre-malignant and malignant colonic epithelial cells. *Eur. J. Cancer*, 34: 1091–1098, 1998.
18. Wahl, A. F., Donaldson, K. L., Fairchild, C., Lee, F. Y., Foster, S. A., Demers, G. W., and Galloway, D. A. Loss of normal p53 function confers sensitization to taxol by increasing G2/M arrest and apoptosis. *Nat. Med.*, 2: 72–79, 1996.
19. Simizu, S., Takada, M., Umezawa, K., and Imoto, M. Requirement of caspase-3(-like) protease-mediated hydrogen peroxide production for apoptosis induced by various anticancer drugs. *J. Biol. Chem.*, 273: 26900–26907, 1998.
20. Spiegel, R. J. The acute toxicities of chemotherapy. *Cancer Treat. Rev.*, 8: 197–205, 1981.