Eur J Cancer Clin Oncol, Vol. 22, No. 7, pp. 883–889, 1986.
Printed in Great Britain.

0277–5379/86$3.00+0.00
Pergamon Journals Ltd.

Analysis of Nuclear m-AMSA Content by DNA Fluorochrome Competition*

BORJE S. ANDERSSON,† MILOSLAV BERAN, BARTHEL BARLOGIE, NGUYEN T. VAN‡ and KENNETH B. McCREDIE

Departments of Hematology and ‡Laboratory Medicine, University of Texas, M.D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Avenue, Houston, TX 77030. U.S.A.

Abstract—*Spectrofluorometry and flow cytometry were used to measure cellular uptake of the anti-leukemic drug 4′-(9-acridinylamino) methane sulfon-m-anisidide (m-AMSA). Because of its very low intrinsic fluorescence, we used m-AMSA to quench DNA-fluorescence induced by the vital DNA fluorochrome Hoechst 33342. Maximum fluorescence was obtained with cells incubated in the fluorochrome alone. Subsequent incubation of cells in increasing concentrations of m-AMSA resulted in a gradual decrease in fluorescence. Upon incubation in drug-free medium, the quenching phenomenon was reversible, consistent with rapid exit of m-AMSA from the cells. The novel competitive fluorescence assay for cellular uptake for m-AMSA showed a better correlation to nuclear accumulation of the drug, than to its overall cellular accumulation, which may be important in assessment of cellular resistance to m-AMSA, with possible low nuclear accumulation of the drug. When this competitive fluorescence technique for measurement of cellular m-AMSA concentration was applied in flow-cytometric setting, subpopulations of normal human white blood cells were detected with distinctly different fluorescence patterns, indicating differences in cellular m-AMSA uptake. The potential use of this technique is to detect differences between cell subpopulations with different drug uptake abilities.*

INTRODUCTION

THE DRUG 4′-(9-acridinylamino) methane sulfon-m-anisidide (m-AMSA), is an acridine derivative with intercalating properties [1]. It induces complete remission in 20–30% of adult patients with acute myelogenous leukemia in relapse when a cumulative dose of more than 450 mg/m^2 of body surface area is reached [2–5]. When combined with Ara-C for the therapy of newly diagnosed leukemia, m-AMSA produces response rates as high as similar regimens containing adriamycin [6]. *In vivo*, m-AMSA appears to be rapidly metabolized, as assessed with ^{14}C-labeled drug and on the basis of ethyl acetate extraction and fluorescence measurements [7–10] or with high-pressure liquid chromatography [11]. The use of such methods for cellular pharmacologic studies will provide information on average drug uptake per cell; the minimum number of cells needed for these analyses is usually in the order of 1–3 × 10^6 per sample. Therefore, cellular heterogeneity for drug uptake cannot be recognized. Possible mechanisms of drug resistance involve decreased uptake and increased efflux, as has been shown for daunorubicin [12,13]. On the other hand, cellular uptake of m-AMSA was similar in sensitive and resistant P-388 murine leukemia cells; however, nuclear accumulation of the drug was decreased, possibly resulting in diminished binding to DNA [14]. Theoretically, a better way for assessment of target cell pharmacology of m-AMSA in relation to its cytotoxic action would, therefore, be the analysis of DNA-bound drug.

The introduction of flow cytometry has made the rapid analysis of cellular properties and events in single cells possible [15–18]. This methodology has also recently been used to quantitate the concentration of anthracyclines in normal hemopoietic and leukemic cells, utilizing the inherent fluorescent properties of the tetracyclic moiety of the anthraquinone glycosides [19–22]. Since m-AMSA itself is very weakly fluorescent, a different approach for the analysis of its cellular uptake by flow cytometry is necessary. Based on the recent

Accepted 13 December 1985.
*This study was supported in part by Grants CA-16672 and CA-28771 from the National Cancer Institute, and The National Institute of Health, Bethesda, Maryland, U.S.A.
Correspondence to: Borje S. Andersson, MD., Ph.D., U.T.M.D. Anderson Hospital, Department of Hematology, 6723 Bertner Avenue, Houston, Texas 77030, U.S.A.
†Special Fellow of the Leukemia Society of America, Inc.

observation of adriamycin-induced fluorescence quenching of the vital DNA-dye Hoechst 33342 [23], we examined the feasibility of measuring m-AMSA cellular uptake and DNA binding by means of Hoechst dye competition, using spectrofluorometry and flow cytometry.

MATERIALS AND METHODS

Cell line

All *in vitro* drug exposure studies were carried out in the human myeloid leukemia cell line HL-60 that was kindly provided from Dr R Gallo's laboratory at the National Cancer Institute, Bethesda, MD [24]. The HL-60 cell line was grown in Iscove's modification of Dulbecco's medium (IMDM, K.C. Biologicals, Lexington, KY) supplemented with 10% fetal calf serum (FCS). Exponentially growing cells (> 95% viability) were used throughout all experiments.

Drugs

m-AMSA (courtesy of Investigational Drug Branch; Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) was dissolved in anhydrous *N,N*-dimethylacetamide at 50 mg/ml and then diluted to 5 mg/ml in 35.3 mM L-lactic acid. The drug was further diluted in distilled water to 200–1000 μg/ml (stock solution) and kept frozen at −80°C until used.

We used ^{14}C-labeled m-AMSA [courtesy of The Stanford Research Institute (SRI-International) Menlo Park, CA, through the Developmental Therapeutics Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD] that was labeled in the $^{C}9$ position and had a specific activity of 19.6 mCi/mol. The radiochemical purity was 98% as determined by thin-layer chromatography. Radiolabeled m-AMSA was used as a tracing element added at 0.6–0.8 μCi/mg of unlabeled drug in some drug-uptake studies.

Hoechst 33342 (Calbiochem–Behring Corporation, LaJolla, CA) was dissolved at 100 μg/ml in phosphate-buffered saline (PBS pH 7.2, GIBCO Laboratories, Grand Island, NY) and kept frozen at −80°C until use. Purified sucrose, used for the gradient as described below was obtained from Bethesda Research Laboratories (Bethesda, MD). It was dissolved to 0.3 M and 0.15 M in double-distilled water.

Preparation of nuclei from HL-60 cells

Nuclei were isolated as has recently been described by gentle detergent lysis [25,26]. Briefly, after a wash in nuclear buffer [26] [NaCl 150 mM, KH_2PO_4 1 mM, $MgCl_2$ 5mM, dithiotreitol 0.1 mM (Sigma Chemical Co., St Louis, MO), pH 6.4], the cells were resuspended on ice in nuclear buffer supplemented with 0.3% Triton X-100 (Sigma Chemical Co., St Louis, MO) and left for 10 min on ice, with gentle agitation every 30 sec. After pelleting at 150 ***g*** for 10 min, the pellet was resuspended in nuclear buffer. Each preparation of nuclei was examined using phase contrast microscopy and bright field microscopy after trypan blue staining to assure that > 95% of the preparation consisted of nuclei and not intact whole cells. The nuclei suspensions were made immediately prior to drug exposure in each experiment.

Hoechst dye quenching experiments

Calf thymus DNA, Type I (purchased from Sigma Chemical Company, St Louis, MO) was extracted 3 times with isopropyl alcohol and chloroform (1 : 3) to reduce contaminating protein to less than 0.5%. In the quenching experiments, the DNA was dissolved in PBS at 20 μg/ml, Hoechst dye was added, 2 or 0.5 μg/ml for 20 min; and subsequently m-AMSA was added in increasing concentrations for 30 min at 37°C prior to spectrofluorometric measurement. Experiments with whole cells were carried out in the following way: 2×10^6 cells/ml in PBS with 5% FCS were incubated with Hoechst 33342 for 60 min at 37°C. After a brief wash and resuspension in warm (37°C) PBS with 5% FCS the cells were incubated for another 60 min with m-AMSA prior to fluorescence measurements. In pilot experiments, cells had been incubated with m-AMSA prior to the fluorochrome. The fluorescence intensities were virtually identical, regardless of the sequence of m-AMSA and Hoechst dye addition. After exposure of cells to the dye and drug, cells were kept on ice until analyzed.

DRUG UPTAKE STUDIES WITH ^{14}-C-LABELED m-AMSA

Whole cells

m-AMSA was diluted in double-distilled water and PBS to desired final concentrations and then mixed with the HL-60 cells at 2×10^6/ml. After incubation for 60 min at 37°C, duplicate samples of 0.5 ml (10^6 cells) were withdrawn and layered on top of a double sucrose gradient (0.5 ml each of 0.15 and 0.3 M sucrose in water) [27]. The cells were spun down for 15 sec at 15,600 ***g*** in an Eppendorff Model 5414 Microcentrifuge and the tubes were immediately decanted. After resuspending the pellet in 1 ml PBS and sonicating for 15 sec on a Heat Systems-Ultrasonics, Inc. (Plainview, NJ) Model W-220-F sonicator, the suspension was transferred to glass liquid scintillation vials. Ten milliliters of Liquiscint (National Diagnostics Co., Somerville, NY) were added and the cell-bound radioactive drug was measured by counting over 10 min in a liquid scintillation spectrometer with

90% counting efficiency. The average amount of m-AMSA taken up per cell was calculated from the mean value of duplicate samples as compared to a standard curve, derived from counting serial dilutions of m-AMSA in PBS alone.

Nuclei

m-AMSA was diluted in double-distilled water and nuclei buffer to desired final concentration and then mixed with nuclei to 2×10^6 nuclei/ml. After incubating for 15 min (steady state for m-AMSA uptake, data not shown), duplicate samples of 0.8 ml were layered on top of 0.8 ml of 0.15 M sucrose. Further handling was as for whole cells and the average nuclear uptake of m-AMSA was calculated as described above.

Spectrofluorometry

Spectrofluorometric assays were carried out in a Perkin–Elmer MPF-44A spectrofluorometer. The wavelengths of excitation and emission were 355 and 450 nm, respectively, corresponding to the excitation and emission maxima for Hoechst 33342.

Flow cytometry

The flow cytometric measurements were carried out on a Coulter Electronics EPICS V flow cytometer equipped with a 5W Argon ion laser with ultraviolet capabilities. The laser was tuned at 355 nm at least 30 min prior to each experiment, and the calibration of the EPICS V was performed with fluorescent 10 μm polystyrene beads (Coulter Electronics Inc., Marietta, GA). Fluorescence above 408 nm was detected with a 408-nm-long pass filter (Schott Optimal Glass, Inc., Duryea, PA). Low-angle forward light scatter was used to measure cell size and to gate out cell debris. The flow rate was set at 200–300 cells/sec, and a total of 10,000 cells were analyzed in each sample. Histograms were generated using the multiparameter data acquisition display system (MDADS™ Coulter Electronics Inc.)

RESULTS

Initial experiments were carried out to determine the optimum Hoechst dye concentration both for DNA in solution and for whole cells. At 5 μg/ml, the ratio of DNA-related fluorescence to background was maximal. However, the relative decrease in fluorescence induced by m-AMSA was the same with Hoechst 33342 ranging from 0.2 to 0.5 μg/ml. Figure 1 illustrates the dose-dependent decrease in Hoechst 33342 fluorescence when increasing concentrations of m-AMSA were mixed with cell-free calf thymus DNA. When the analogous experiment was carried out with HL-60 cells in suspension, a similar decline in fluorescence was

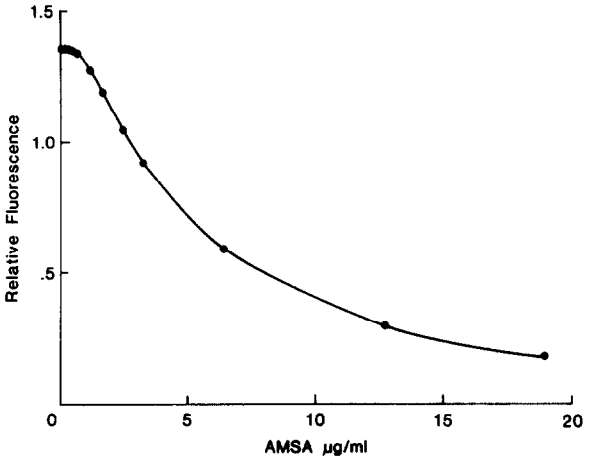

Fig. 1. m-AMSA-related quenching of the Hoechst 33342 (2 μg/ml) *induced DNA fluorescence. The concentration of DNA was* 20 μg/ml. *Excitation was at* 355 nm *and emission at* 450 nm.

observed, however, with a steeper initial part of the slope (Fig. 2). Figure 3 examines the cellular and nuclear uptake of radiolabeled m-AMSA. The cellular and nuclear uptake curves for m-AMSA are quite different with only a fraction of m-AMSA being taken up by the nuclei as compared to whole cells. The relationship between cellular fluorescence after exposure to Hoechst 33342 (0.5 μg/ml) and m-AMSA, and nuclear/cellular uptake of ^{14}C-labeled drug is shown in Figs. 4 and 5. The relation between nuclear drug accumulation and the cellular decrease in fluorescence is close to a straight line and deviates significantly from the straight line only at a high concentration of m-AMSA (20 μg/ml), whereas the relationship between total cell

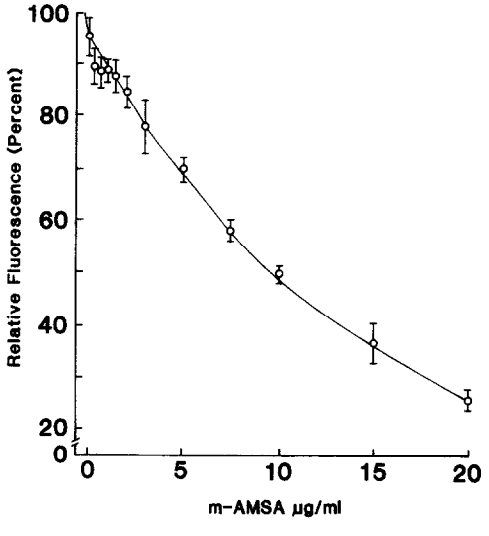

Fig. 2. m-AMSA related quenching of Hoechst induced DNA fluorescence in HL-60 cells. The cells (2×10^6/ml) *were incubated in Hoechst* 33342 (0.5 μg/ml) *for* 60 min *at* 37°C *and after a brief wash, they were incubated in m-AMSA at increasing concentrations for* 60 min *prior to analyzing the fluorescence (see also Materials and Methods). Each point is the mean of three separate experiments* (bar, ± S.D.).

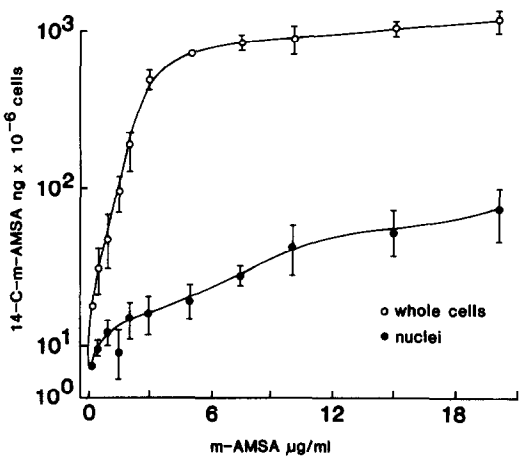

Fig. 3. Uptake of ^{14}C-m-AMSA in whole cells (○) and nuclei (●) of HL-60 leukemia cells. Each point is the mean of three separate experiments (bar, ± S.D.).

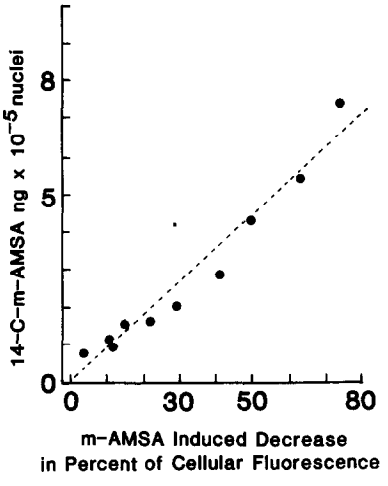

Fig. 4. The correlation between cellular fluorescence and nuclear uptake of ^{14}C-m-AMSA. The data points were obtained from the experiments in Figs 2 and 3.

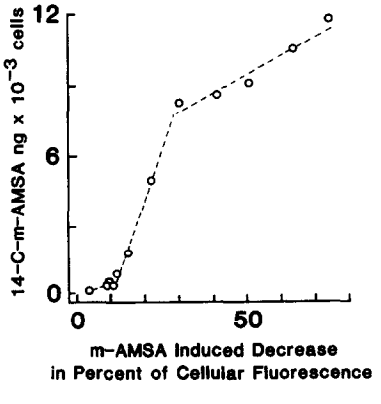

Fig. 5. The correlation between cellular fluorescence and cellular uptake of ^{14}C-m-AMSA. The data points were obtained from the experiments in Figs 2 *and* 3.

uptake of m-AMSA and decrease in cellular fluorescence is more complex (Fig. 5).

To test the competitive binding of m-AMSA and Hoechst 33342 to DNA, m-AMSA-treated HL-60 cells were thoroughly washed in 10 vol. of PBS and resuspended in PBS prior to the addition of Hoechst dye. There was only minimal quenching of Hoechst dye fluorescence when compared to controls incubated with Hoechst dye alone. The almost complete reversibility of the quenching phenomenon suggests effective removal of m-AMSA from DNA-binding sites.

With increasing time intervals between cell procurement and treatment with m-AMSA plus Hoechst 33342, the fluorescence intensity decreased. However, the resulting slopes of the fluorescence-quenching curves were unchanged.

This indicates that the uptake kinetics of Hoechst 33342 may change with *in vitro* storage of the cells, whereas that of m-AMSA was not influenced. In further experiments, the cellular fluorescence patterns were examined immediately after incubation of the cells in Hoechst 33342 or after a subsequent delay during which the cells were kept on ice up to 4 hr prior to the addition of m-AMSA, or the cells were kept on ice up to 4 hr after incubation in both Hoechst dye and m-AMSA. In none of these cases was there any appreciable change in fluorescence that could be attributed to the storage on ice.

In further experiments, normal human peripheral blood mononuclear cells (density < 1.080 g/cm^3, predominantly G_1 population) were incubated with m-AMSA and Hoechst 33342, as described above, and the fluorescence was analyzed with an EPICS V flow cytometer. Figure 6 displays the dose-dependent progressive left shift in fluorescence intensity with increasing m-AMSA concentration. The flow cytometric analysis revealed a pronounced quenching in fluorescence intensity when cells were exposed to concentrations of 0–15 µg/ml of m-AMSA, whereas an increase in drug concentration from 15 to 20 µg/ml had little further effect on the fluorescence. Two-parameter analysis (light scatter or cell size vs fluorescence) in the presence of 15 µg/ml of m-AMSA revealed 2 cell populations with distinctly different fluorescence and light-scatter properties (Fig. 7). The smaller subpopulation of larger cells demonstrated a higher degree of Hoechst dye quenching, consistent with higher intracellular levels of m-AMSA.

DISCUSSION

Using radioisotope technology as a standard, our studies demonstrated the feasibility of a competitive fluorescence assay for measuring m-AMSA binding both to cell-free DNA and its uptake into nuclear DNA of normal white blood cells and in

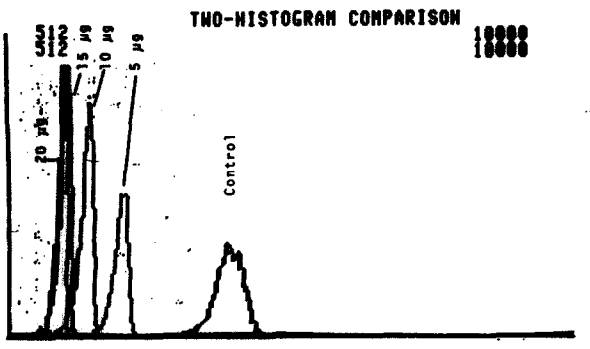

Fig. 6. After incubation of normal peripheral mononuclear white blood cells (density ≤ 1.080 gm/cm³) for 60 min in Hoechst 33342 (0.5 μg/ml), the cells were incubated in m-AMSA at increasing concentrations (5, 10, 15 and 20 μg/ml). The control cells were incubated in Hoechst 33342 alone. The abscissa displays relative fluorescence and the ordinate relative number of cells.

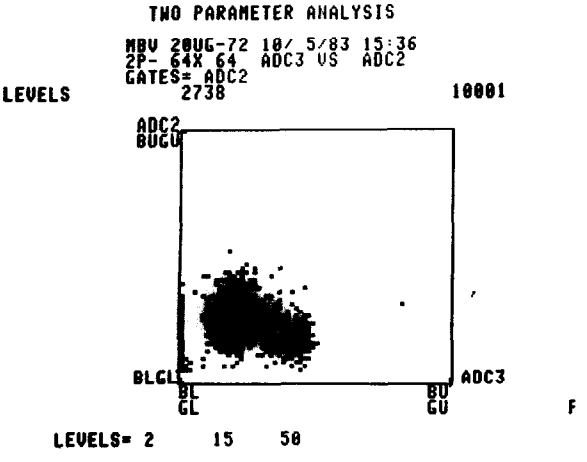

Fig. 7. Two-dimensional expression, cell size (ordinate) vs fluorescence (abscissa) of the experiment in Fig. 6. The concentration of m-AMSA here was 15 μg/ml. Two distinct cell populations with different uptake of drug can be seen.

cultured human myeloid HL–60 cells. Spectrofluorometry permits analysis of the mean cellular DNA uptake of m-AMSA while flow cytometry measures cellular m-AMSA uptake more rapidly and in individual cells. In comparison to spectrofluorometry, flow cytometry can be performed on much smaller samples (4×10^6 vs $3–5 \times 10^5$ cells), and possible cellular heterogeneity for m-AMSA uptake can be appreciated.

Most studies utilizing flow cytometric analysis of cellular drug uptake utilized the fluorescent properties of anthracycline antibiotics themselves [19–22]. However, since daunorubicin and adriamycin-related fluorescence is quenched upon binding of these drugs to DNA [28], much of the fluorescence measured by such direct assays possibly originates from the cytoplasmic compartment and from drug that has entered the nucleus, albeit not yet bound to DNA. Consequently, the direct fluorescence method might predominantly measure unbound drug and hence under estimate the biologically relevant fraction of drug bound to the critical target, DNA. An advantage of the present competitive fluorescence assay is that it probably preferentially analyzes the amount of DNA-bound drug rather than the total cellular uptake of m-AMSA, as indicated by comparison of the gradual decrease in fluorescence to increasing doses of m-AMSA to the corresponding uptake of ^{14}C-labeled drug in nuclei and whole cells respectively (Figs 4 and 5).

An increase in active cellular efflux of adriamycin and daunorubicin has been invoked as one mechanism of acquired cellular resistance to those drugs [12,13,29,30]. However, a recent study on the development of cellular m-AMSA resistance in murine P-388 leukemia showed the overall cellular drug uptake to be similar in drug-resistant and sensitive cells, whereas there was a decrease in nuclear translocation of the drug [14]. By relying on an indicator DNA-reactive fluorochrome, the present competition assay appears to measure DNA-bound rather than overall drug uptake. This property of our assay offers an advantage above previous methods when resistance may result from defects in nuclear translocation rather than overall cellular drug uptake. The reversibility of Hoechst 33342 quenching after thorough cell washing is in agreement with previous observations of rapid efflux of m-AMSA from drug-loaded cells transferred to drug-free medium [31].

One of the most interesting aspects of our study was the observation of nonuniform m-AMSA uptake into normal human mononuclear cells. When both cell size and fluorescence were measured concurrently, two distinct populations with different drug uptake were recognized. We cannot exclude the possibility that this difference was due to the cell cycle-related differences in DNA content. However, greater than 97% of all cells had a uniform G_1 DNA content as determined independently on fixed cells, using ethidium bromide and mithramycin [16]. Hence, the difference in Hoechst dye quenching most likely reflects differences in m-AMSA uptake by different hemopoietic cells, which has previously been noted in normal bone marrow cells using a direct fluorescence assay for daunorubicin [21].

Cell separation experiments to obtain purified cell subpopulations would be required to demonstrate differences in cellular drug uptake directly. Thus, flow cytometry is a promising method of studying the heterogeneity of cellular drug pharmacology with regards to differences in drug resistance. When coupled with radioisotope technology and cell sorting, differences in fluorescence can be directly related to results obtained with standard technology. When applied to fluorescent drugs such as anthracyclines, the combined analysis of

direct fluorescence and Hoechst dye quenching may permit the concurrent assessment of total or cytoplasmic, as well as nuclear, drug concentration.

Acknowledgment — The authors gratefully acknowledge the assistance of Mrs Carolyn Davis for the typing of this manuscript.

REFERENCES

1. Cain BF, Baguely BC, Denny WA. Potential antitumor agents. 28. Deoxyribunucleic acid polyintercalating agents. *J med Chem* 1978, **21**, 658–668.
2. Legha SS, Keating MJ, Zander AR, McCredie KB, Bodey GP, Freireich EJ. 4′-(9-acridinylamino) methanesulfon-m-anisidide (AMSA). A new drug effective in the treatment of adult acute leukemia. *Ann Intern Med* 1980, **93**, 17–21.
3. Legha SS, Keating MJ, McCredie KB, Bodey GP, Freireich EJ. Evaluation of AMSA in previously treated patients with acute leukemia. Results of therapy in 109 adults. *Blood* 1982, **60**, 484–490.
4. Slevin ML, Shannon MS, Prentice HG, Goldman AJ, Lister TA. A phase I and II study of m-AMSA in acute leukemia. *Cancer Chemother Pharmac* 1981, **6**, 137–140.
5. Lawrence HJ, Ries CA, Reynolds RD, Lewis JP, Kortez MM, Torti FM. AMSA-A promising new agent in refractory acute leukemia. *Cancer Treat Rep* 1982, **66**, 1475–78.
6. McCredie KB, Keating MJ, Estey EH, Zander AR, Bodey GP, Drewinko B, Freireich EJ. Use of 4′-(9-acridinylamino) methane-sulfon-m-anisidide (AMSA) cytosine arabinoside (Ara-C) Vincristine–prednisone combination (AMSA-OAP) in poor risk patients in acute leukemia. *Proc Am Ass Cancer Res* 1981, **22**, 479.
7. Cysyk RL, Shoemaker D, Adamson RH. The pharmacologic disposition of 4′-(9-acridinylamino)-methane sulfon-m-anisidide in mice and rats. *Drug Metab Dispos* 1977, **5**, 579–590.
8. Shoemaker D, Ayers O, D'Anna M, Cysyk R. Effect of diethyl-maleate and phenobarbitrol on the metabolism and biliary excretion of 4′-(9-acridinylamino) methane sulfon-m-ansidide (m-AMSA) (NSC-141549) in rats. *Proc Am Ass Cancer Res* 1979, **20**, 280.
9. Hall SW, Benjamin RS, Legha SS, Gutterman JU, Loo TL. Clinical pharmacokinetics of the new antitumor agent AMSA. *Proc Am Ass Cancer Res* 1979, **20**, 175.
10. Hall SW, Friedman J, Legha SS, Benjamin RS, Gutterman JU, Loo TL. Human pharmacokinetics of a new acridine derivative 4′-(9-acridinylamino) methane sulfon-m-anisidide (NSC 249992). *Cancer Res* 1983, **43**, 3422–3426.
11. Jurlina JL, Paxton JW. High performance liquid chromatographic method for the determination of 4′-(9-acridinylamino) methanesulfon-m-anisidide in plasma. *J Chromat* 1983, **276**, 367–374.
12. Dano K. Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim biophys Acta* 1973, **323**, 466–483.
13. Dano K. Cross-resistance between vinca-alkaloids and anthracyclines in Ehrlich ascites tumor *in vivo*. *Cancer Chemother Rep* 1972, **56**, 701–708.
14. Kessel D, Wheeler C, Chou TH, Howard WS, Johnson RK. Studies on a mode of resistance to m-AMSA. *Biochem Pharma* 1982, **31**, 3008–3010.
15. VanDilla MA, Trujillo TT, Mullaney PE, Coulter JR. Cell microfluorometry: a method for rapid fluorescence measurement. *Science (Wash)* 1969, **163**, 1213–1214.
16. Barlogie B, Spitzer G, Hart J, Johnston D, Buchner T, Schumann J, Drewinko B. DNA-histogram analysis of human hemopoietic cells. *Blood* 1976, **48**, 245–258.
17. Tobey RA, Crissman HA. Use of microfluorometry in detailed analysis of chemical agents on cell cycle progression. *Cancer Res* 1972, **32**, 2726–2732.
18. Krishan A, Pitman SW, Tattersall MHN, Paika KD, Smith DC, Frei E, III. Flow microfluorometric pattern of human bone marrow and tumor cells in response to cancer chemotherapy. *Cancer Res* 1976, **36**, 3813–3820.
19. Krishan A, Ganapathis R. Laser flow cytometry and cancer chemotherapy: detection of intracellular anthracyclines by flow cytometry. *J Histochem Cytochem* 1979, **27**, 1655–1656.
20. Krishan A, Ganapathis R. Laser flow cytometric studies on the intracellular fluorescence of anthracyclines. *Cancer Res* 1980, **48**, 3895–3900.
21. Sonneveld P, Van Den Eng GJ. Differences in uptake of adriamycin and daunorubicin by normal BM cells and acute leukemia cells determined by flow cytometry. *Leukemia Res* 1981, **5**, 251–257.
22. Durand RE, Olive PL. Flow cytometry studies of intracellular Adriamycin in single cells *in vitro*. *Cancer Res* 1981, **41**, 3489–3494.
23. Preisler HD. Alteration of binding of the supravital dye Hoechst 33342 to human leukemic cells by adriamycin. *Cancer Treat Rep* 1978, **62**, 1393–1396.
24. Gallagher R, Collins S, Trujillo J, McCredie KB, Ahearn M, Tsai S, Aulakh GS, Ting R, Ruscetti F, Gallo R. Characterization of the continuously differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia. *Blood* 1979, **54**, 254–268.
25. Filipski J, Kohn KW. Ellipticine-induced protein-associated DNA breaks in isolated L 1210 nuclei. *Biochim biophys Acta* 1982, **698**, 280–286.

26. Pommier Y, Schwartz RE, Kohn KW, Zwelling LA. Formation and rejoining of deoxyribonucleic acid double-strand breaks induced in isolated cell nuclei by antineoplastic intercalating agents. *Biochemistry* 1984, **23**, 3194–3201.
27. Andersson B, Beran M, Stuckey S, McCredie KB, Mavligit GM. Sensitivity and resistance to chemotherapy in acute leukemia: correlation with *in vitro* drug uptake and lack of potentiation by verapamil. Submitted for publication.
28. Trouet A, Deprez de Campeneere D, deDuve C. Chemotherapy through lysosomes with a DNA–daunorubicin complex. *Nature* (*New Biol*) 1972, **239**, 110–112.
29. Skovsgaard T. *In vitro* and *in vivo* study of cross-resistance between daunorubicin and daunorubicin–DNA complex in Ehrlich ascites tumor. *Cancer Chemother Pharmac* 1979, **2**, 43–47.
30. Aldrich CD. Pleiotropic phenotype of cultured murine cells resistant to maytansine, vincristine, colchicine and adriamycin. *JNCI* 1979, **63**, 751–757.
31. Zwelling LA, Michaels S, Erickson LC, Ungerleider RS, Nichols M, Kohn KW. Protein-associated deoxyribonucleic acid strand breaks in L 1210 cells treated with the deoxyribonucleic acid intercalating agents 4′-(9-acridinylamino) methanesulfon-m-anisidide and adriamycin. *Biochemistry* 1981, **20**, 6553–6563.