Cancer Chemother Pharmacol (1996) 39: 157–161

SHORT COMMUNICATION

Manlio Tolomeo · Rosa Alba Gancitano · Maurizio Musso
Ferdinando Porretto · Rosanna Perricone
Vincenzo Abbadessa · Aurelio Cajozzo

Comparative activity of idarubicin and idarubicinol in combination with cyclosporin A in multidrug-resistant leukemia cells

Received 7 April 1995/Accepted 13 May 1996

Abstract 4-Demethoxydaunorubicin (idarubicin, IDA) is an anthracycline that has shown good cytotoxic activity in vitro against tumor cell lines displaying the multidrug-resistant (MDR) phenotype. IDA is converted in the liver into idarubicinol (2HIDA) and, in this form, seems to exert its antitumoral activity in vivo. Recent studies have shown that 2HIDA has tumoricidal activity similar to that of the parent drug when tested in vitro in sensitive neoplastic cells. In this work we compared in vitro the effects of IDA and 2HIDA used alone and in combination with 2 μ*M* cyclosporin A (CyA) in the MDR leukemic cell lines FLCR and K562R and in their sensitive parent cell lines FLC and K562. IDA and 2HIDA showed the same cytotoxic activity in sensitive cells. After 1 h of exposure of cells to each anthracycline, we observed that the cellular uptake of IDA and 2HIDA was also similar. In resistant cells, 2HIDA was 3–4 times less active than IDA. We observed that the intracellular uptake of 2HIDA was lower than that of IDA, and this may be correlated with a greater ability of P-glycoprotein to expel 2HIDA as opposed to IDA. Indeed, when MDR cells were exposed to IDA and 2HIDA in combination with 2 μ*M* CyA, the cytotoxic effect of these anthracyclines was the same, and it was similar to that observed in sensitive cells. These data confirm the utility of the combination of IDA and an MDR-reversing agent in hematological malignancies displaying the MDR phenotype.

Key words: Idarubicin · Idarubicinol · Cyclosporin A · Multidrug resistance

This work was supported by CNR and AIRC

M. Tolomeo (✉) · R.A. Gancitano · M. Musso · F. Porretto · R. Perricone · V. Abbadessa · A. Cajozzo
Chair of Hematology, University of Palermo, Via del Vespro 129, I-09 127 Patermo, Italy

Introduction

Idarubicin (IDA) is an anthracycline that has shown superior in vitro cytotoxicity against tumor cell lines as compared with daunorubicin (DNR) [5, 15]. A low degree of cross-resistance was observed in tests against multidrug-resistant (MDR) tumor cell lines in vitro [12, 18]. The major circulating metabolite of IDA is the alcohol product of ketoreductase biotransformation, dihydro- idarubicin (2HIDA) or idarubicinol. Following the administration of IDA to leukemic patients, systemic exposure to 2HIDA is greater than that to IDA, suggesting that it may have a role in the antitumoral activity of IDA [17]. In vitro 2HIDA has tumoricidal activity that is similar to that of the parent drug and greater than that of other anthracycline alcohol metabolites (daunorubicinol and doxorubicinol) [9]. In this work we demonstrated that 2HIDA is less active than IDA in MDR cells, but when they are used in combination with a resistance-modifying agent (RMA), their activity is similar. The RMA used in this study was cyclosporin. A (CyA). We chose CyA because it is a potent modulator of MDR in vitro and has been shown to be active in reversing drug resistance in animal models. The concentration of CyA that is required to reverse MDR in vitro is about 2 μ*M*. In preliminary clinical trials, serum CyA levels of 2 μ*M* were achieved in 90% of patients with acceptable toxicity when this RMA was given in combination with etoposide [10, 21].

Materials and methods

Cell cultures

The erythroleukemia cell lines FLC and K562 and their MDR counterparts FLCR and K562R were used in this study. Cells were grown in suspension culture using RPM1 1640 medium containing 10% fetal calf serum (FCS) and 1% penicillin and streptomycin. The

sensitive and resistant cell lines have a similar doubling time (18 h for FLC and FLCR, 25 h for K562 and K562R), and they demonstrate no erythroid differentiation as defined by negative benzidine staining. The assay conducted by flow cytometry using the fluorescein-labeled C219 monoclonal antibody. (P-glycoCHEKTM C-219) showed an expression of P-glycoprotein only in resistant cell lines.

Cytotoxicity assays

Tumor-cell cytotoxicity assays were conducted by seeding 2×10^5 cells in 1 ml of complete medium in 16-mm wells (tissue-culture cluster-24 wells, Costar). IDA or 2HIDA at concentrations ranging between 0.002 and 0.8 μg/ml, in the presence or absence of 2 μ*M* CyA, were added immediately there after, and cells were incubated at 37 °C in an atmosphere containing CO_2. After 48 h for FLC and FLCR and 72 h for K562 and K562R, the numbers of cells were evaluated using an Ortho ELT-8/WS counter (Ortho Diagnostic System Inc.). Cell growth inhibition was expressed as a percentage of control proliferation.

Flow cytometry

For flow-cytometry determination of intracellular anthracycline accumulation, a FACScan device (Becton Dickinson) equipped with an argon laser using a 488-nm line operating at 15 mW was used. Light was collected through a 545-nm filter for IDA and 2HIDA intracellular accumulation studies.

IDA and 2HIDA intracellular accumulation studies

Cells (1×10^6) were incubated with either IDA or 2HIDA at concentration ranging between 0.025 and 3 μg/ml in the presence or absence of 2 μ*M* CyA. After 60 min of incubation at 37 °C in an atmosphere containing 5% CO_2, cells were washed twice in phosphate-buffered saline (PBS) and then resuspended in drug-free complete medium. IDA and 2HIDA fluorescence was immediately measured using the FACScan device with the appropriate filter.

Intracellular IDA and 2HIDA distribution studies

The intracellular localization of IDA and 2HIDA was studied by ultraviolet illumination using a Leitz Orthoplan fluorescence microscope equipped with a superpressure mercury lamp and a 545-nm filter.

Evaluation of the resistance index and MDR-reversal index

The resistance index (RI) to IDA and 2HIDA was calculated by the formula

$$RI = IC_{50} MDR\ cells / IC_{50}\ sensitive\ cells,$$

and the resistance-reversal index (RRI) was calculated by the formula

$$RRI = IC_{50}\ sensitive\ cells / IC_{50}\ MDR\ cells + RMA,$$

where IC_{50} *MDR cells* + *RMA* represents the concentration of IDA or 2HIDA, in the presence of 2 μ*M* CyA, capable of inhibiting the growth of resistant cells by 50%.

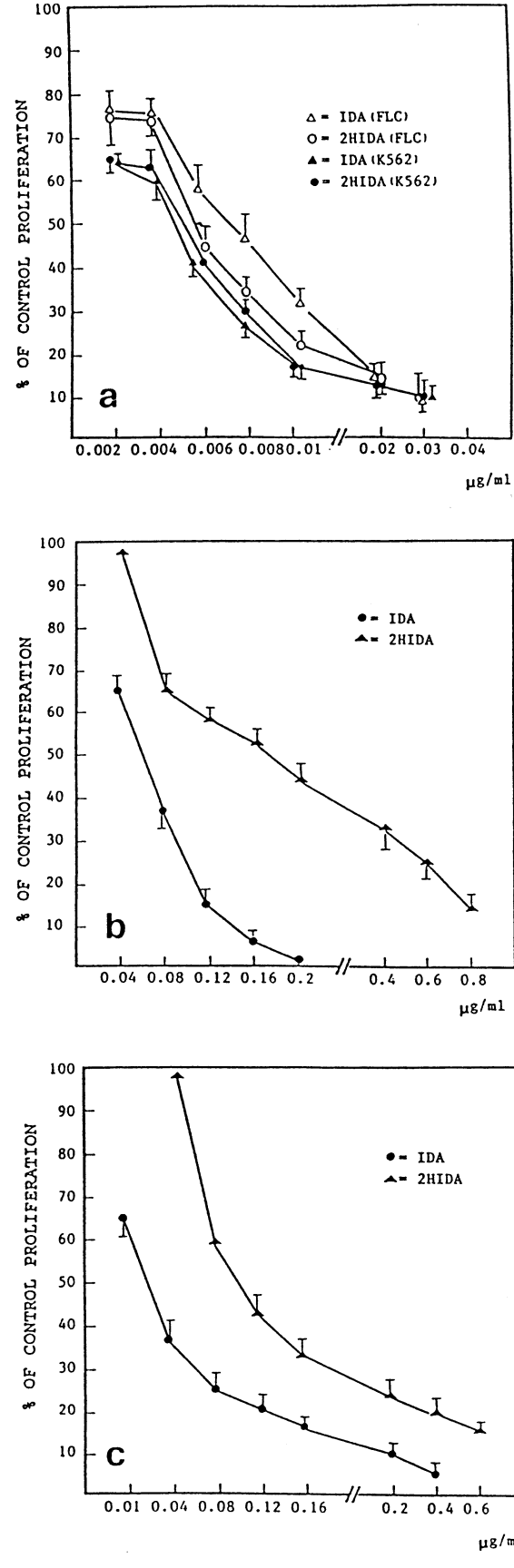

Fig. 1 a–c Antiproliferative activity of IDA and 2HIDA in **a** sensitive FLC and K562 cells, **b** resistant FLCR cells, and **c** resistant K562R cells

Results

In sensitive FLC and K562 cells, IDA showed a level of cytotoxicity similar to that of its metabolite 2HIDA (Fig. 1a). An important difference between these anthracyclines was observed in resistant cell lines; in both FLCR and K562R cells the cytotoxic activity of IDA was greater than that of 2HIDA (Fig. 1b, c). The IC_{50} values and the RI of these drugs are summarized in Table 1.

Table 1 RIs to IDA and 2HIDA as determined in FLCR and K562R cell lines

Cells	IC_{50} IDA (μg/ml)	RI	IC_{50} 2HIDA (μg/ml)	RI
FLC	0.007		0.005	
		8.57		36
FLCR	0.06		0.18	
K562	0.005		0.005	
		5		20
K562R	0.025		0.1	

FLCR and K562R lines showed a low degree of resistance to IDA; the RI to 2HIDA was 4 times greater than that to IDA in both resistant cell lines. The IC_{50} 2HIDA: IC_{50} IDA ratio showed that IDA was 3 and 4 times more active than 2HIDA in the FLCR line and in K562R cells, respectively.

Figures 2A and 2B show the cytotoxicity of IDA and 2HIDA used in combination with 2 μ*M* CyA in resistant cells as compared with that of these anthracyclines used alone in sensitive cell lines. CyA was capable of completely reversing IDA and 2HIDA resistance, and the RRI was 1 in both resistant cell lines. Therefore, 2 μ*M* CyA increased by 36 and 20 times, respectively, the cytotoxic activity of 2HIDA in FLCR and K562R cells but enhanced only 8.57 and 5 times the cytotoxic activity of IDA in the respective cell lines. The ratio between IC_{50} 2HIDA and IC_{50} IDA changed from 3–4 to 1 when CyA was associated with each anthracycline. CyA (2 μ*M*) alone did not modify the growth of resistant cells,

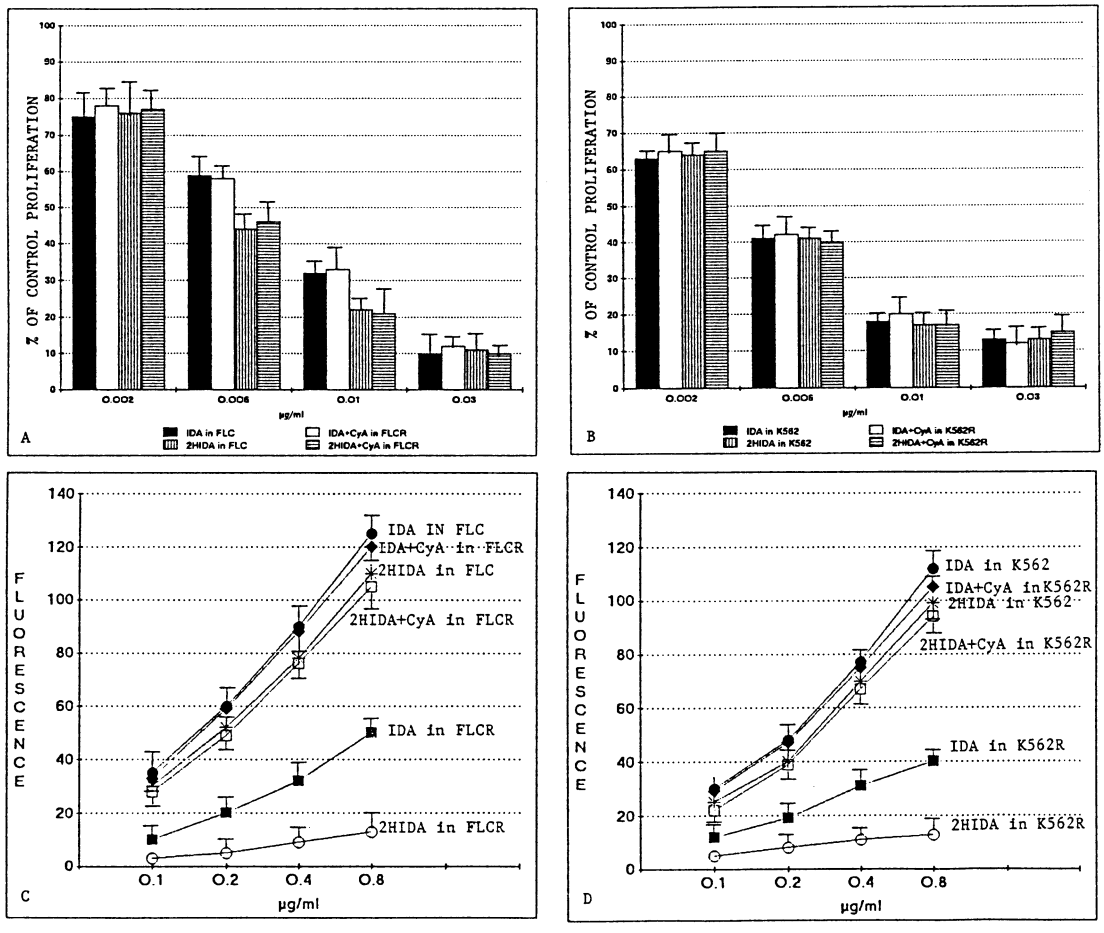

Fig. 2 A–D Antiproliferative activity of IDA and 2HIDA in combination with 2 μ*M* CyA in resistant **A** FLCR **B** K562R cell lines. Comparisor with the antiproliferative activity of IDA and 2HIDA alone in sensitive cell lines. **C**, **D** IDA and 2HIDA uptake by senstive and resistant cells as determined in the presence and absence of 2 μ*M* CyA

and no significant difference in cytotoxicity was observed in sensitive cells exposed to each anthracycline alone or in combination with 2 μ*M* CyA.

There was no important difference in IDA or 2HIDA uptake by sensitive FLC or K562 cells. In resistant cells the uptake of IDA was greater than that of 2HIDA, and this difference was more evident in the FLCR line. When resistant cells were exposed to IDA or 2HIDA in combination with 2 μ*M* CyA, the uptake of each anthracycline was similar to that observed in the respective sensitive parent cell line exposed to each anthracycline alone (Figs. 2c, 2d).

No difference in intracellular fluorescence distribution was observed in sensitive cell lines exposed to IDA and 2HIDA. For both anthracyclines the fluorescence was located prevalently in the nucleus, showing a uniform pattern of distribution, and in the Golgi region and lysosomes. In resistant cells the pattern of fluorescence distribution after exposure to IDA or 2HIDA was similar to that seen in sensitive cells, but the intensity of fluorescence was lower. The only difference observed between these anthracyclines in resistant cells was a higher intensity of fluorescence after exposure to IDA.

Discussion

IDA has been reported to have greater cytotoxic activity in vitro than other anthracyclines. For example, the cytotoxic activity of IDA has been demonstrated to be 7.6 times greater than that of doxorubicin (DXR) and 12.3 times greater than that of epirubicin (EPR) in C6 glioblastoma cells [15], 7.7 and 11 times greater than that of DNR and DXR in the LoVo cell line [5], and 2 and 2.5 times greater than that of DNR and DXR in the lymphoblastic leukemia cell line CCRF-CEM [9]. The difference is more evident in MDR cell lines. In the C6 0.5 E resistant clone, IDA is 27.8 and 38.8 times more active than DNR and DXR, respectively [15], and in the resistant LoVo/Dx cell line it is 64.2 and 64 times more active respectively [6].

However, many clinical studies have shown no important increase in the response rate after administration of IDA to patients with refractory malignancies [1–4, 13, 20]. The discrepancy between the in vitro and in vivo results obtained with IDA in refractory leukemias may be related, at least in part, to the pharmacokinetic properties of IDA. Speth et al. [17] have shown that IDA is metaboilized in the liver to an active metabolite, idarubicinol (2HIDA), which attains plasma levels higher than those of the parent compound. Following the administration of IDA to adult or pediatric patients, systemic exposure to 2HIDA is 2–12 times greater than that to IDA [14].

In this study we observed that the ctotoxic activity of IDA and 2HIDA was similar in sensitive FLC and K562 leukemia cells, where as in FLCR and K562R MDR cells, 2HIDA was 3–4 times less active than IDA. Intracellular accumulation of IDA was greater than that of 2HIDA in resistant cells and was similar in sensitive parental cells, suggesting that the difference observed in cytotoxic activity between IDA and 2HIDA in resistant cells can be correlated with a difference in the uptake of these anthracyclines.

By fluorescence microscopy we observed differences only in fluorescence intensity between sensitive and resistant cells exposed to IDA or 2HIDA. The fluorescence in sensitive cells was distributed diffusely in both the nucleus and the cytoplasm. In contrast, resistant cells showed less fluorescence in the nucleus and cytoplasm. No important difference in intracellular distribution was observed. Other authors have shown a prevalent nuclear fluorescence in sensitive cells as compared with MDR cells after exposure to DNR or Adriamycin [7, 16]. However, IDA and 2HIDA are more active than these anthracyclines in resistant cells, and this may explain, at least in part, the similar intracellular distribution observed in sensitive and resistant cells.

The cytotoxic activity and the rate of cell uptake of IDA and 2HIDA become similar when these anthracyclines are used in combination with an RMA. We observed that 2 μ*M* CyA could completely reverse the resistance to these anthracyclines in FLCR and K562R cells. The cytotoxicity and cell drug-uptake data obtained with IDA and 2HIDA in combination with 2 μ*M* CyA in resistant cells were analogous with those recorded for these anthracyclines as single agents in sensitive cells. Since IDA has good activity in resistant cells, its combination with CyA results in an increase in cytotoxic activity of only 5–8 times. In contrast, when 2HIDA is combined with CyA, the cytotoxic activity of 2HIDA in resistant cells was increased 20–36 times.

Previous studies have shown that RMAs, such as verapamil or CyA, are capable of reversing DNR and Adriamycin resistance in MDR cells, but a complete reversal of resistance to these anthracyclines occurs only rarely [8, 11, 19]. When a complete reversal of DNR and Adriamycin resistance was achieved, the cytotoxic activity of these anthracyclines was at least similar to that observed in sensitive parent cells and was lower than the cytotoxic activity obtained with IDA or 2HIDA. In conclusion, these data suggest the utility of the IDA and CyA combination in hematological malignancies displaying the MDR phenotype.

Acknowledgements We are grateful to Pharmacia for the generous gift of idarubicin and idarubicinol and to Maria Grandi, Pharmacia Research Institute for helpful discussion during the course of this study.

References

1. Baer MR, Christiansen NP, Frankel SR, Brunetto VL, Mròzek K, Bloomfield CD, Herzig GP (1993) High-dose cytarabine, idarubicin, and granulocyte colony-stimulating factor remission induction therapy for previously untreated de novo and secondary adult acute myeloid leukemia Semin Oncol 6 [Suppl 8]: 6
2. Berman E, Heller G, Santorsa J, Meckenzie S, Gee TS, Kempin S, Gulati S, Andreeff M, Kolitz J, Gabrilove J, Reich L, Mayer K. Keefe DF, Trainor K, Schluger A, Penenberg D, Raymond V, O'Reilly R, Jhanwar S, Young C, Clarkson B (1991) Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorbuicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia. Blood 77: 1666
3. Carella AM, Santini G, Martinengo M, et al (1985) 4-Demethoxydaunorubicin in refractory or relapsed acute leukemias. A pilot study. Cancer 55: 1452
4. Daghestani AN, Arlin ZA, Leyland-Jones B, Gee TS, Kempin SJ, Mertelsmann R, Budman D, Schulman P, Baratz R, Williams L, Clarksom BD, Young CW (1985) Phase I and II clinical and pharmacological study of 4-demethoxydaunorubicin (idarubicin) in adult patients with acute leukemia Cancer Res 45: 1408
5. Dodion P, Sanders C, Rombaut W, Mattelaer MA, Rozencweig M, Stryckmans P, Kenis Y (1987) Effect of daunorubicin, carminomycin, idarubicin and 4-demethoxydaunorubicinol against human normal myeloid stem cells and human malignant cells in vitro. Eur J Clin Oncol 23: 909
6. Facchetti I, Grandi M, Cucchi P, Geroni C, Penco S, Vigevani A (1991) Influence of lipophilicity on cytotoxicity of anthracyclines in LoVo and LoVo/Dx human cell lines. Anticancer Drug Des 6: 385
7. Hindenburg AA, Gervasoni JE, Krishana S, Stewart VJ, Rosado M, Lutzky J, Bhalla K, Baker MA, Taub RN (1989) Intracellular distribution and pharmacokinetics of daunorubicin in anthracycline-sensitive and -resistant HL-60 cells. Cancer Res 49: 4607
8. Hu XF, Martin TJ, Bell DR, Luise M, de Zalcberg JR (1990) Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines. Cancer Res 50: 2953
9. Kuffel MJ, Reid JM, Ames MM (1992) Anthracyclines and their C-13 alcohol metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture. Cancer Chemother Pharmacol 30: 51
10. Lum BL, Kaubisch S, Yahanda AM, et al (1992) Alteration of etoposide pharmacokineics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. J Clin Oncol 10: 1635
11. Nooter K, Oostrum R, Jonker R, Dekken H, Stokdijk W, Engh G (1989) Effect of cyclosporin A on daunorubicin accumulation in multidrug-resistant P388 cells measured by real-time flow cytometry. Cancer Chemother Pharmacol 23: 296
12. Petrini M, Mattii L, Valentini P, Sabbatini ARM, Grassi B, Grandi M (1993) idarubicin is active on MDR cells: evaluation of DNA synthesis inhibition on P388 cell lines. Ann Hematol 67: 227
13. Rassman SMB, Turker A, Powles RL, Smith AG, Newland AC, Erskine JG, Pearce RM, Goldstone AH (1993) Idarubicin for remission induction of acute myeloid leukemia: United Kingdom multicenter experience. Semin Oncol 6 [Suppl 8]: 13
14. Reid JM, Pendergrass TW Krailo MD, Hammond GD, Ames MM (1990) Plasma pharmacokinetics and cerebrospinal fluid concentrations of idarubicin and idarubicinol in pediatric leukemia patients. Cancer Res 50: 6525
15. Schott B, Robert J (1989) Comparative cytotoxicity, DNA synthesis inhibition and drug incorporation of eight anthracyclines in a model of doxorubicin-sensitive and resistant rat glioblastoma cells. Biochem Pharmacol 38: 167
16. Schuurhuis GJ, Broxterman HI, Cervantes A, Heijningen THM van, Lange JHM, de Baak JPA, Pinedo HM, Lankelma J (1989) Quantitative determination of factors contributing to doxorubicin resistance in multidrug-resistant cells. J Natl Cancer Inst 81: 1887
17. Speth PAJ, Loo FAJ, van de Linssen PCM, Wessels HMC, Haanen C (1986) Plasma and human leukemic cell pharmacokinetics of oral and intravenous 4-demethoxydaunomycin Clin Pharmacol Ther 40: 643
18. Tolomeo M, Luparello M, Caravello E, Perricone R, Abbadessa V, Cajozzo A (1991) Effects of epirubicin and idarubicin in multidrug resistant (MDR) Friend leukemia cells (abstract). Proceedings, 11th meeting of the international Society of Haematology–European and African Division, Basel, August 31–September 6, p 137
19. Twentyman PR, Fox NE, White DJG (1987) Cyclosporin A and its analogues as modifiers of Adriamycin and vincristine resistance in a multi-drug resistant human lung cancer cell line. Br J Cancer 56: 55
20. Vogler WR, Velez-Garcia E, Weiner RS, et al (1992) A phase III trial comparing idarubicin and daunorubicin in combination with cytarabine in acute myelogenous leukemia: a Southeasten Cancer Study Group study J Clin Oncol 10: 1103
21. Yahanda AM, Adler KM, Fisher GA, Brophy NA, Halsey J, Hardy RI, Gosland MP, Lum BL, Sikic BI (1992) Phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 10: 1624