Cancer Chemother Pharmacol (1995) 36: 368–372

ORIGINAL ARTICLE

Pankaj B. Desai · Renu Bhardwaj · Bharat Damle

Effect of tamoxifen on mitoxantrone cytotoxicity in drug-sensitive and multidrug-resistant MCF-7 cells

Received: 4 February 1994/Accepted: 30 December 1994

Abstract The influence of the antiestrogen tamoxifen (TAM) on the activity of mitoxantrone (MXN), was evaluated against wild-type MCF-7/WT and their multidrug-resistant variant MCF-7/ADR cells. Multidrug resistance (MDR) in this cell line which was selected for resistance to Adriamycin (ADR), is associated with increased expression of P-glycoprotein (P-gp). In a clonogenic assay it was observed that TAM (1–10 μM) significantly enhanced the activity of MXN in the MCF-7/ADR but not in the drug-sensitive cell line. Isobologram analysis indicated that the effect of the combination was additive in the parental MCF-7/WT cells and strongly synergistic in the MDR MCF-7/ADR cells. Also, TAM (10 μM) caused a three-fold increase in the steady-state levels (C_{ss}) of MXN in MCF-7/ADR cells but did not modulate MXN levels in MCF-7/WT cells. The observed synergism in MCF-7/ADR cells was perhaps due to the increase in C_{ss} of MXN that may involve interaction of TAM with P-gp. The combination of MXN and TAM may be useful in the treatment of drug-sensitive and drug-resistant breast cancer.

Key words Mitoxantrone · Tamoxifen · Multidrug resistance

Introduction

The anthracycline antibiotic doxorubicin or Adriamycin (ADR) is one of the most active drugs in the treatment of metastatic breast carcinomas [27, 33]. However, the incidence of cumulative dose-dependent cardiotoxicity and the occurrence/development of drug resistance severely limit the usefulness of this drug [29, 35]. MXN, an anthracenedione, is a promising antitumor compound under phase III clinical investigations in the treatment of advanced metastatic breast carcinoma [17, 19, 23]. Several studies have indicated that MXN is less cardiotoxic than ADR, and may be useful in treating patients with compromised cardiac function as a substitute for the anthracycline [2, 26]. However, tumor cells that develop multidrug resistance (MDR) following treatment with anticancer drugs such as ADR are often cross-resistant to MXN [11, 15, 18, 24]. MDR is most often associated with overexpression of an efflux protein called P-glycoprotein (P-gp) [29], but other variations including changes in the levels of DNA topoisomerase II and glutathione-*S*-transferase (GST) have also been reported [13, 18]. It appears that P-gp plays an important role in acquired MDR in refractory advanced breast cancer. This is evidenced by high levels of the MDR1 gene that encodes P-gp (and the mRNA associated with P-gp) observed in patients treated with chemotherapeutic drugs [16, 24].

The clinical response rate to MXN is significantly lower in patients with prior treatment with ADR compared to those without previous exposure to drug therapy [19, 23]. This suggests that acquired drug resistance restricts the activity of MXN against breast cancer. Tamoxifen (TAM), a nonsteroidal antiestrogen, is used as a first line of treatment of estrogen receptor-positive breast cancers. The low incidence of side effects observed with TAM in the treatment of advanced breast cancer makes it a drug of choice for adjuvant therapy [6, 20]. Interestingly, TAM also appears to be a promising MDR-reversing agent [4, 8, 20, 28]. It is suggested that TAM inhibits P-gp-mediated drug efflux from MDR cells [4, 8]. In a preliminary study, we investigated the cytotoxic activity of the combination of MXN and TAM against breast cancer MCF-7/WT cells and their MDR variant MCF-7/ADR cells. The interaction of the two compounds was evaluated using

P.B. Desai (✉) · R. Bhardwaj · B. Damle
Division of Medicinal Chemistry and Pharmaceutics, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209-0470, USA

isobologram analysis and the steady-state cellular MXN accumulation/retention in the presence and the absence of TAM was also investigated.

Materials and methods

Drugs and chemicals

MXN.2HCl was a gift from Lederle Laboratories (Pearl River, N.Y.). TAM citrate was purchased from Sigma Chemical Co. (St. Louis, Mo.). Cell culture medium RPMI 1640, fetal bovine serum, penicillin G and streptomycin, glutamine and trypsin were purchased from GIBCO BRL (Gaithersburg, Md.). All other miscellaneous chemicals and solvents were purchased from commercial sources.

Cell lines

Parental wild-type MCF-7/WT and MDR MCF-7/ADR cells were provided by Dr. K. H. Cowan (National Cancer Institute, NIH, Bethesda, Md.). MCF-7 cells are estrogen receptor-positive cells (58,900 binding sites/cell) [10, 22]. MCF-7/ADR is associated with a loss of estrogen receptors [36]. In addition to elevated levels of P-gp, MCF-7/ADR cells overexpress the anionic isozyme of GST (GSTπ) as well as glutathione peroxidase and altered levels of DT-diaphorase [1, 30]. Both cell lines were grown in RPMI-1640 supplemented with 10%, fetal bovine serum, 20 units/ml penicillin G, 20 µg/ml streptomycin and 3.5 m*M* glutamine. In the case of MCF-7/ADR cells, the culture medium was also supplemented with 500 ng/ml ADR. Drug-containing medium was replaced with fresh drug-free medium 7 days before the initiation of the experiment. Cells were grown as monolayer cultures and maintained at 37°C in an incubator with a humidified atmosphere of 5% CO_2 in air.

Colony formation assay

Cytotoxicity was assessed using a colony-formation assay. Logarithmically growing MCF-7/WT cells (400/ml) and MCF-7/ADR cells (500/ml) were plated in triplicate onto 60 × 15 mm plastic petri dishes containing 5 ml complete medium. Cells were seeded, depending on the plating efficiency, to yield 200–300 colonies in control plates. MXN (1–50 n*M* in the case of MCF-7/WT cells; 0.1–1 µ*M* in the case of MCF-7/ADR cells) and/or TAM (1–10 µ*M*) were added 24 h later. Following a drug treatment period of 6 h, the drug-containing medium was removed, the cells were washed with phosphate-buffered saline solution (PBS) and fresh medium was added. Cultures were further incubated for a period of 10–12 days and then stained with (2%) methylene blue solution in methanol. The number of colonies containing 50 or more cells was determined. Dose response of the survival of cells with respect to control was plotted against MXN concentration.

Median effect analysis

Survival curves obtained from clonogenic assays were analyzed using median effect analysis as suggested by Chou and Talalay [9]. The median effect equation is shown below:

$$F_a/F_u = (D/D_m)^m \quad (1)$$

where, D is the dose, F_a and F_u are the fraction of cells affected and unaffected (survived the drug treatment), D_m is the dose required to produce the median effect (analogous to IC_{50}) and m is the Hill coefficient that determines the sigmoidicity of the curve. The logarithmic transformation of the Eq. 1 yields the following linear relationship:

$$\log(F_a/F_u) = m\log(D) - m\log(D_m) \quad (2)$$

Thus, a plot of $\log(F_a/F_u)$ versus log(drug concentration) yields an x-intercept [where $\log(F_a/F_u) = 0$, the value of F_a/F_u is 1] that corresponds to D_m, or IC_{50}. The effect of TAM on MXN activity was evaluated by the determination of an enhancement index (EI) as shown below:

$$EI = \frac{IC_{50} \text{ of MXN in the absence of TAM}}{IC_{50} \text{ of MXN in the presence of TAM}}$$

Isobologram analysis

The nature of the MXN and TAM interaction was evaluated as described by Berenbaum [3]. The combination of MXN and TAM was considered homergic since they both exhibited cytotoxicity. Cells were treated with MXN alone, TAM alone or the combination of the two agents at several concentrations of TAM (1–10 µ*M*). IC_{50} values were determined as described. For each cell line IC_{50} isoboles were constructed to determine whether the combination of the drugs was additive, synergistic or antagonistic.

Cellular drug accumulation

Exponentially growing cells (1×10^6) were plated in triplicate onto 100 × 20 mm plastic petri dishes in 15 ml medium and incubated for 72 h. MXN (2 µg/ml) in the presence and the absence of TAM (10 µ*M*) was then added. At different time intervals (15, 30, 45, 60 120, 240 and 360 min) after the drug treatment, the drug containing medium was removed, and the cells trypsinized and harvested. Following centrifugation, the cell pellets were washed thrice with 2 ml ice-cold PBS (0.1 *M*; 0°C). Cells were recentrifuged and then 2 ml of ethanolic HCl supplemented with ascorbic acid (5% w/v) was added to the pellet. The cells were disrupted using a sonicator and the resulting suspension was recentrifuged. The concentration of the extracted drug in the supernatant was determined using HPLC. Cell debris was resuspended and an aliquot (100 µl) was withdrawn for protein determination using the Bradford method [5]. Standard curves for MXN concentration were obtained by similarly treating controls and adding known amounts of MXN to the cell pellets before extraction and drug assay. The solvent extraction was observed to yield complete recovery of MXN.

HPLC analysis of MXN

A previously reported method with modifications was utilized for the HPLC analysis of MXN [25]. The HPLC apparatus consisted of a model 2350 solvent delivery system (ISCO), model V^4 variable wavelength absorbance detector (ISCO) and a C_{18} Spherisorb reverse-phase column (dimensions 4.6 × 250 cm, particle size 5 µm). MXN was eluted isocratically at ambient temperature with a 45:55 combination of acetonitrile acetate buffer (pH 3.0, 0.01 *M*) at a flow rate of 0.8 ml/min. MXN was detected at 662 nm at an AUFS of 0.01 with a sensitivity of 10 ng/ml. The retention time was 4.6 min.

Statistical analysis

Student's t-test was used for determining the significance of the observed differences, taking $P < 001$ as the level of significance, unless otherwise stated.

Results

Cytotoxicity studies

First, the survival of MCF-7/WT and MCF-7/ADR cells treated with MXN (0–1 μ*M*) was examined. A comparison of the IC_{50} values, obtained from the median effect plots, indicated that the MCF-7/ADR cells were 36-fold resistant to MXN compared to the MCF-7/WT cells. The effect of several concentrations of TAM (1–10 μ*M*) on the sensitivity of cells to MXN was also examined using a colony formation assay. Figure 1 shows the survival of the cells treated with a range of MXN concentrations with or without the presence of TAM (10 μ*M*). A significant ($P < 0.01$) enhancement in the activity of MXN in the presence of TAM was observed in the case of MCF-7/WT cells (Fig. 1A) as well as in the case of MCF-7/ADR cells (Fig. 1B). For all the dose response curves shown, the median effect plots (not shown) were observed to have a correlation coefficient of 0.97 or greater. The EIs indicated that TAM (10 μ*M*) caused a 9-fold and a 25-fold increase in the activity of MXN in MCF-7/WT and in MCF-7/ADR cells, respectively. Table 1 lists the IC_{50} values of MXN and the EIs in the presence of several concentrations of TAM. A significant ($P < 0.01$) potentiation of MXN activity was observed against drug-sensitive as well as drug-resistant cells at all TAM concentrations employed. The degree of potentiation in the case of the resistant cells was, however, markedly higher than that in the wild-type parental cells.

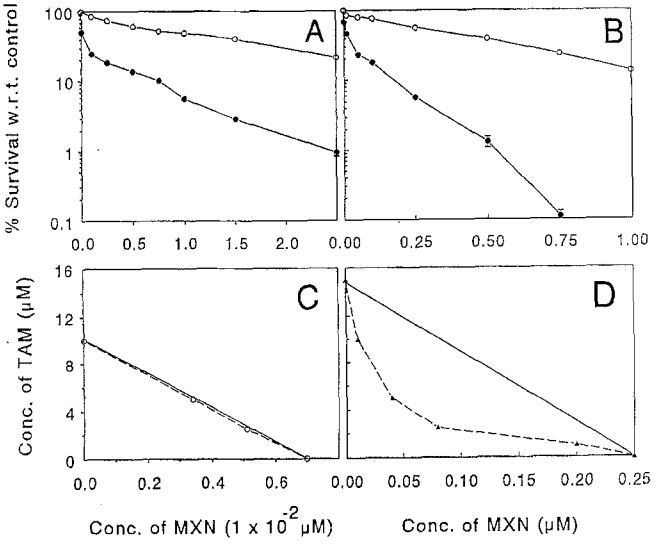

Fig 1A–D. The influence of TAM on the survival of drug-sensitive and MDR MCF-7 cells evaluated using a colony formation assay. **A, B** Survival of MCF-7/WT (**A**) and MCF-7/ADR (**B**) cells treated with the indicated concentration of MXN and TAM (10 μ*M*). (○ MXN alone, ● MXN + TAM). Each point represents the mean of triplicate observations. *Bars* represent SD (shown where larger than symbol size). **C, D** Isobologram analysis of the survival data. The IC_{50} isoboles were obtained for cells treated with MXN alone, TAM alone or MXN ± TAM (1–10 μ*M*). The *solid line* joining the IC_{50} of each agent represents the expected isobole if the two compounds interacted additively. The *dotted curves* indicate the observed isoboles for the combination of the two agents in MCF-7/WT (**C**) and MCF-7/ADR cells (**D**)

Table 1 Summary of the effects of several concentration of TAM and MXN towards the two cell lines. The IC_{50} values are means of triplicate observations (*EI*, enhancement index)

TMX Concentration (μ*M*)	MCF-7/WT		MCF-7/ADR	
	MXN IC_{50} (μ*M*)	EI	MXN IC_{50} (μ*M*)	EI
0	0.007	–	0.25	–
1	0.006	1.2	0.20	1.3
2.5	0.005	1.4	0.10	2.6
5.0	0.003	2.3	0.03	9.0
10.0	0.001	8.8	0.01	25.0

Isobologram analysis

At concentrations greater than 5 μ*M*, TAM alone was cytotoxic to both cell lines. As shown in Fig. 1A and B, TAM (10 μ*M*) by itself lowered the survival of MCF-7/WT cells to 53% and of MCF-7/ADR cells to 68% of the control value. We examined the nature of the interaction between MXN and TAM using isobologram analysis. Fig. 1C and D show the IC_{50} isoboles obtained for survival of MCF-7/WT and MCF-7/ADR cells. The solid line joining IC_{50} of MXN alone (plotted on the *x*-axis) and TAM alone (plotted on the *y*-axis) for each cell line represents the expected isobole of additivity. If MXN and TAM interacted additively, then the IC_{50} values of the combination would be represented by this line. For MCF-7/WT cells the observed IC_{50} isobole for MXN and TAM combinations (dotted line) almost completely coincides with this line of additivity (Fig. 1C). On the other hand, the IC_{50} values of MXN in MCF-7/ADR cells in the presence of several concentrations of TAM were well below the additivity line (Fig. 1D). Thus, it appears that TAM modulated the activity of MXN in the resistant cells in a synergistic manner and additively in the case of the drug-sensitive cells.

Cellular MXN accumulation

Figure 2 shows the accumulation of MXN by MCF-7/WT and MCF-7/ADR cells over a period of 6 h. Resistance to MXN in the MDR variants was accompanied by a 4.8-fold reduction in the net intracellular steady-state drug levels. TAM (10 μ*M*) did not significantly ($P < 0.01$) alter the MXN levels in the drug-sensitive cells (Fig. 2A). However, there was a significant ($P < 0.01$) increase in the drug levels in MCF-7/ADR cells incubated with MXN in the

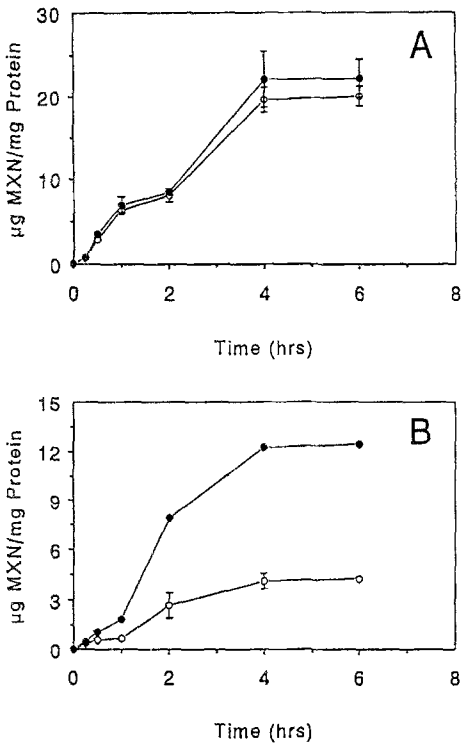

Fig 2A,B. Intracellular MXN levels in MCF-7/WT (**A**) and MDR MCF-7/ADR cells (**B**). Exponentially growing cells were treated with MXN (2 μg/ml) with or without TAM (10 μ*M*). Intracellular MXN levels were determined as a function of time as described in the Methods (○ cells treated with MXN alone, ● cells treated with MXN + TAM). Each point represents the mean of triplicate observations. *Bars* represent SD (shown where larger than symbol size).

presence of TAM at all time points of measurements (Fig. 2B). For example, TAM (10 μ*M*) caused a three-fold increase in the C_{ss} of MXN.

Discussion

This study demonstrated that TAM significantly enhanced the activity of MXN in MCF-7/ADR cells. The nature of the interaction of the two agents appeared to be additive in the parent cell line MCF-7/WT and synergistic in the MDR cells. This difference in the mode of interaction was accompanied by a marked increase in the levels of MXN in the MDR cells but not in the drug-sensitive cells. Since the two cell lines differ in the expression of P-gp, the synergistic potentiation of MXN activity in MCF-7/ADR cells may involve interaction of TAM with P-gp. The mechanism of modulation of MXN resistance by TAM is not clear. It may involve competitive inhibition of P-gp, inhibition of calmodulin or protein kinase C [4, 8]. These possibilities are currently under investigation in several laboratories. It is interesting that, compared to MCF-7 cells, MCF-7/ADR cells were somewhat resistant to TAM (approximately 1.6-fold, Fig. 1C and D). This could be related to the altered estrogen receptor status of MCF-7/ADR cells [36] or it may suggest that TAM is a substrate for P-gp-mediated efflux. In this regard, it is worthwhile to note that TAM has been shown to prevent the binding of [^{3}H]-azidopine to P-gp [22].

Conventionally used doses of TAM (10–20 mg daily) result in steady-state levels of approximately 0.4–1.0 μ*M*. However, several studies have shown that higher levels can be achieved without serious toxicities. For example, Trump et al. employed TAM at doses of 150 mg/m^2 which resulted in a mean plasma drug level of 4 μ*M* without dose-limiting toxicities [34]. In some other clinical studies, high doses of TAM have been employed that result in serum levels in the range of 3–8 μ*M* [7, 32]. Although at TAM concentrations > 5 μ*M* a synergistic interaction between MXN and the antiestrogen was observed in this study, Leonessa et al. have reported that at these concentrations a TAM and doxorubicin combination in MCF-7/ADR cells is antagonistic [22]. Thus it appears that while the clinical utility of TAM levels above 5 μ*M* is not clear, concentrations in the range of 4–5 μ*M* can be achieved safely. The interaction of TAM employed at concentrations of 2.5 and 5 μ*M* with MXN observed in this study is, therefore, clinically relevant. Moreover, it is important to note that the metabolites of TAM, *N*-desmethyl TAM and 4-OH-TAM have also been shown to have "anti-MDR" activity [21]. The plasma levels of *N*-desmethyl TAM are known to approach or exceed those of the parent compound [20]. It is likely, therefore, that the sum of the concentrations of TAM and its metabolite(s) may be in the range of 5–10 μ*M* and these compounds can synergistically modulate drug resistance in vivo.

The role of P-gp in resistance to MXN is not well understood. MDR cells derived by treating parental cells with MXN as a primary selecting agent do not overexpress P-gp. However, cells with elevated levels of P-gp that are derived by treatment with other agents such as ADR and etoposide are often cross-resistant to MXN [11, 14, 24]. This has been observed in several cell lines used in our laboratory including MCF-7/ADR [12]. The poor response rate associated with MXN therapy in patients with prior treatment with ADR suggests that acquired cross-resistance between ADR and MXN is a limiting factor in the use of MXN for the treatment of breast cancer [19, 23]. Therefore, efforts to potentiate the activity of MXN in drug-resistant tumors are important. The two cell lines used in this study constitute a clinically relevant model for preliminary evaluation of the combination of MXN and TAM. MCF-7/WT is an estrogen receptor-positive cell line established from a malignant pleural effusion in a female patient with metastatic breast cancer [31]. MDR variant MCF-7/ADR cells were obtained by stepwise treatment with ADR [14].

In conclusion, the combination of MXN and TAM could have two advantages. First, whether estrogen receptor- positive cells are MXN-resistant or sensitive, simultaneous treatment with this DNA topoisomerase II inhibitor and the antiestrogen could be expected, at

the least, to be additive. Second, the activity of MXN in drug-resistant tumors can be synergistically potentiated by TAM because of its "anti-P-gp" activity. The combination of several chemotherapeutic agents with TAM and its analog toremifene are currently being evaluated in several estrogen receptor-positive and-negative MDR cell lines.

References

1. Batist G, Tulpule A, Sinha BK, Katki AG, Myers CE, Cowan KH (1986) Overexpression of a novel anionic glutathione transferase in multidrug resistant human breast cancer cells. J Biol Chem 261: 15544
2. Benjamin RS, Chawla SP, Ewer MS, Carrasco H, Mckay B, Holmes F (1985) Evaluation of mitoxantrone cardiac toxicity by nuclear angiography and endomyocardial biopsy: an update. Invest New Drugs 3: 117
3. Berenbaum MC (1981) Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 35: 269
4. Berman E, Adams M, Duigou-Osterndorf R, Godfrey L, Clarkson B, Andreeff M (1991) Effect of tamoxifen on cell lines displaying multidrug resistance phenotype. Blood 77: 818
5. Bradford M (1976) A rapid and sensitive method for quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72: 248
6. Buckley MT, Goa KL (1989) Drug evaluation: tamoxifen-A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic uses. Drugs 37: 451
7. Cantwell B, Carmichael J, Millward MJ, Chatterjee M, Harris AL (1989) Intermittent high dose tamoxifen (HDT) with oral etoposide (EPO): phase I and II clinical studies. Br J Cancer 8: 450
8. Chaterjee M, Harris AL (1990) Reversal of acquired resistance to adriamycin in CHO cells by tamoxifen and 4-hydroxytamoxifen: role of drug interaction with alpha-1 acid glycoprotein. Br J Cancer 62: 712
9. Chou TC, Talalay T (1984) Quantitative analysis of dose effect relationship: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22: 27
10. Clarke R, Currier S, Kaplan O, Lovelac E, Boulay V, Gottesman MM, Dickson RB (1992) Effect of P-glycoprotein expression on sensitivity to hormones in MCF-7 human breast cancer cells. J Natl Cancer Inst 84: 1506–1512
11. Dalton WS, Durie BGM, Alberts DS, Gerlach JH, Cress AE (1988) Characterization of a new drug-resistant human myeloma cell line that expressed P-glycoprotein. Cancer Res 46: 5125
12. Damle BD, Rajagopalan S, Desai PB (1994) Dipyridamole modulates multidrug resistance and intracellular as well as nuclear levels of doxorubicin in B16 melanoma cells. Int J Cancer 56: 113
13. Deffie AM, Alam T, Seneviratne C, Beenken SW, Batra JK, Shea TC, Henner WD, Goldenberg GJ (1988) Multifactorial resistance to adriamycin: relationship of DNA repair, glutathione transferase activity, drug efflux, and P-glycoprotein in cloned cell lines of adriamycin-sensitive and-resistant P388 leukemia. Cancer Res 48: 3595
14. Fairchild CR, Ivy SP, Kao-Shan C, Peng-Whang J, Rosen M, Israel MA, Melera PW, Cowan KH, Goldsmith ME (1987) Isolation of amplified and overexpressed DNA sequences from Adriamycin resistant human breast cancer cells. Cancer Res 47: 5141
15. Gerlach JH, Kartner N, Bell DR, Ling V (1986) Multidrug resistance. Cancer Surv 5: 25
16. Goldstein LJ, Galski H, Fojo AT, Willingham MC, Lai SL, Gazdar A, Piker R, Green A, Crist W, Brodfeur GM, Lieber M, Cossman J, Gottesman MM, Pastan I (1989) Expression of a multidrug resistance gene in human tumors and tissues. J Natl Cancer Inst 81: 116
17. Hainsworth JD, Andrews MB, Johnson DH, Greco FA (1991) Mitoxantrone, fluorouracil and high dose leucovorin: an effective well tolerated regimen for metastatic breast cancer. J Clin Oncol 9: 1731
18. Harker WG, Slade DL, Dalton WS, Meltzer PS, Trent JM (1989) Multidrug resistance in mitoxantrone-selected HL-60 leukemia cells in the absence of P-glycoprotein overexpression. Cancer Res 49: 4542
19. Henderson CI, Allegra JC, Woodcock T, Wolff S, Bryan S, Cartwright K, Dukart G, Henry D (1986) Randomized clinical trials comparing mitoxantrone with doxorubicin in previously treated patients with metastatic breast cancer. J Clin Oncol 4: 672
20. Jordan VC, Robinson SP, Welsons WV (1989) Resistance to antiestrogen therapy. In: D. Kessel (ed) Resistance to antineoplastic drugs. CRC Press, Boca Raton, p 403
21. Kirk J, Houlbrook S, Stuart NSA, Stratford IJ, Harris AL, Carmichael J (1993) Differential modulation of doxorubicin toxicity to multidrug and intrinsically drug resistant cell lines by anti-oestrogens and their major metabolites. Br J Cancer 67: 1189–1195
22. Leonessa F, Jacobson M, Boyle B, Lippman J, McGarvey M, Clarke R (1994) Effect of tamoxifen on the multidrug-resistant phenotype in human breast cancer cells: isobologram, drug accumulation, and Mr 170,000 glycoprotein (gp 170) binding studies. Cancer Res 54: 441–447
23. Neidhart JA, Gochnour D, Roach R, Hoth D, Young D (1986) A comparison of mitoxantrone and doxorubicin in breast cancer. J Clin Oncol 4: 672
24. Pastan I, Gottesman MM (1991) Multidrug resistance. Annu Rev Med 42: 277
25. Peng YM, Ormberg D, Alberts DS (1982) Improved high performance liquid chromatography of the new antineoplastic agents bisanterene and mitoxantrone. J Chromatogr 233: 235
26. Posner LE, Dukart G, Goldberg J, Bernstein T, Cartwright K (1985) Mitoxantrone: an overview of safety and toxicity. Invest New Drugs 3: 123
27. Powles TJ, Smith IE, Ford HT, Combers RC, Mary J, Gazet JC (1980) Failure of chemotherapy to prolong survival in a group of patients with metastatic breast cancer. Lancet 1: 580
28. Ramu A, Glaubiger D, Fuks Z (1984) Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. Cancer Res 44: 4392
29. Riordan JR, Ling V (1985) Genetic and biochemical characterization of multidrug resistance. Pharmacol Ther 28: 51
30. Sinha BK, Katki AG, Batist G, Cowan KH, Myers CE (1987) Differential formation of hydroxyl radicals by Adriamycin in sensitive and resistant MCF-7 human breast cancer tumor cells: implications for the mechanism of action. Biochem 26: 3776
31. Soule HD, Vazquez J, Long A, Alberts S, Brennan M (1973) A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 51: 1409
32. Tong W, Berman E, Lin S (1992) Pharmacokinetic study of high dose tamoxifen with daunorubicin: a phase I trial with escalation of tamoxifen. Proc Am Assoc Cancer Res 33: 528
33. Tormey DC (1975) Adriamycin (NSC 123127) in breast cancer: an overview of studies. Cancer Chemother Rep 6: 319
34. Trump DL, Smith DC, Ellis PG, Rogers MP, Schol SC, Winer EP, Panella TJ, Jordan VC, FIne RL (1992) High-dose oral tamoxifen, a potential multidrug-resistant-reversal agent: phase I trial in combination with vinblastine. J Natl Cancer Inst 84: 1811–1816
35. Van Hoff DD, Layard MV, Basa P (1979) Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91: 710
36. Vickers PJ, Dickson RB, Shoemaker R, Cowan KH (1988) A multidrug resistant MCF-7 human breast cancer cell line which exhibits cross-resistance to antiestrogens and hormone-independent tumor growth in vivo. Mol Endocrinol 2(10): 886