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Sensitization to Doxorubicin Resistance in Breast Cancer Cell Lines by Tamoxifen and Megestrol Acetate

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ABSTRACT. Acquired drug resistance is a major factor in the failure of doxorubicin-based chemotherapy in breast cancer. We determined the ability of megestrol acetate and/or tamoxifen to reverse doxorubicin drug resistance in a doxorubicin-resistant breast cancer line (the human MCF-7/ADR). The cytotoxicity of doxorubicin, megestrol acetate, and/or tamoxifen was determined in the sensitive and resistant cell lines utilizing the sulphorhodamine B assay. Tamoxifen alone produced an IC_{50} (concentration resulting in 50% inhibition of control growth) of 10.6 μM, whereas megestrol acetate alone resulted in an IC_{50} of 48.7 μM in the MCF-7/ADR cell line. The IC_{50} of doxorubicin in MCF-7/ADR was 1.9 μM. Neither megestrol acetate alone nor tamoxifen alone at 1 or 5 μM altered the IC_{50} of doxorubicin. However, the combination of tamoxifen (1 or 5 μM) and megestrol acetate (1 or 5 μM) synergistically sensitized MCF-7/ADR cells. Additionally, megestrol acetate and tamoxifen inhibited iodoarylazidoprazosin binding to P-glycoprotein, and, in their presence, there was an increased doxorubicin accumulation in the MCF-7/ADR cells. Furthermore, the combination of tamoxifen and megestrol acetate had much less effect on the cytotoxicity of doxorubicin in MCF-7 wild-type cells. Clinically achievable concentrations of tamoxifen and megestrol acetate can *largely* sensitize MCF-7/ADR to doxorubicin. The combination of these three drugs in a clinical trial may be informative. BIOCHEM PHARMACOL 52;7:1097–1102, 1996.

KEY WORDS. tamoxifen; megestrol acetate; adriamycin resistance; breast cancer

Cancer of the female breast is a common malignancy occurring in approximately 10% of women in some populations. Although surgery alone can cure approximately 50% of patients, the impact of adjuvant hormonal therapy or chemotherapy on breast cancer survival has been significant, but adjuvant therapy does not prevent relapse in a substantial number of patients. A significant percentage of patients with breast cancer do relapse, and they ultimately die from metastatic disease. The development of combination chemotherapy has resulted in significant responses in the majority of patients with metastatic breast cancer. However, the median duration of response is usually less than 1 year with median survivals of 18–24 months. A small percentage of responses are long-term, complete remissions that are durable for several years [1–7].

Doxorubicin is the most active agent in the treatment of breast cancer, with a reported objective response rate as high as 47% in previously chemotherapy naive patients with metastatic breast cancer [8]. The development of drug resistance is a major factor responsible for treatment failure. Acquired drug resistance to the anthracyclines is associated with cross-resistance to a large number of compounds that are chemically unrelated. This phenomenon, MDR,# is associated with overexpression of a membrane protein (P-glycoprotein) and other factors [9]. Reversal of MDR has been accomplished by a growing number of agents including verapamil, quinidine, and cyclosporine [10, 11]. Unfortunately, the concentration × time of many of these agents that is necessary to reverse drug resistance is difficult to achieve *in vivo*. Possible mechanisms of reversal of MDR include cell membrane alterations [12] and decreased efflux via competition for binding sites on the P-glycoprotein [13].

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Abbreviations: MDR, multidrug resistance; FBS, fetal bovine serum; MEM, minimum essential medium; SRB, sulphorhodamine B; WT, wild-type; ADR, doxorubicin-resistant; and IAAP, iodoarylazidoprazosin.

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The ability of tamoxifen at 3–8 μM to partially reverse doxorubicin drug resistance associated with MDR has been demonstrated in P388/ADR [14] and, more recently, in the human breast cancer cell line MCF-7/DOX [15]. Moreover, tamoxifen has an IC_{50} of 10 μM in MCF-7/DOX cells [15]. Clinically achievable concentrations of high-dose tamoxifen (120 mg/m^2 p.o. b.i.d.) and its active metabolites are approximately 8 μM [16].

Progesterone at 2–25 μM can partially reverse vinblastine resistance of P-glycoprotein overproducing murine macrophage-like cells. This reversal of resistance is associated with increased vinblastine accumulation [17]. There are no reports of the effects of antiestrogens plus progesteronal agents on reversal of MDR (doxorubicin). The effects of tamoxifen and megestrol acetate on the reversal of doxorubicin drug resistance in breast cancer cells are investigated in this report.

MATERIALS AND METHODS

Drugs and Reagents

Tamoxifen and megestrol acetate were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). In all experiments, desiccated tamoxifen was used. Doxorubicin hydrochloride as a stock isotonic solution of 2 mg/mL was available from a commercial source (Adria Laboratories of Canada Ltd., Toronto, Ontario). Tamoxifen and doxorubicin were stored at 4°. Megestrol acetate was stored at room temperature. Tamoxifen and megestrol acetate were solubilized in 95% (w/v) ethanol HPLC grade before each experiment and freshly used. The final volume of ethanol in treated wells was <0.1%. Control wells were treated with the vehicle (medium and similar amount of ethanol). Each experimental condition was repeated in triplicate in at least two separate experiments. Doxorubicin was protected from light during experiments. Dilutions were made in cell culture medium (RPMI-1640 plus 10% FBS and antibiotics) prior to usage.

Materials

RPMI-1640, FBS, penicillin-streptomycin, MEM nonessential amino acids, MEM sodium pyruvate, trypsin-EDTA, and trypan blue were purchased from Gibco (Grand Island Biological Co., Grand Island, NY, U.S.A.). Hypoxanthine-thymidine was purchased from ICN Biomedicals (ICN BIOMEDICALS Canada Ltd., Ontario). SRB and Tris base were purchased from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).

Cell Culture

WT sensitive and ADR sublines were used in this study. The human breast cancer cell line MCF-7 is a hormonally responsive (estrogen receptor positive) line. The corresponding ADR cells are approximately 50- to 200-fold resistant, and they have been demonstrated to overexpress MDR-1, as well as glutathione-utilizing enzymes. They are estrogen receptor negative, and are sensitized significantly but not completely to doxorubicin by verapamil [18–20]. The human breast carcinoma cell line MCF-7/ADR resistant to doxorubicin was obtained from NCI, DCT Tumor Repository, NCI-FCRF, Frederick, MD, and maintained in RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 2 mg/mL streptomycin, and 3×10^{-6} M doxorubicin. Three passages prior to the experiments, MCF-7/ADR was passaged in drug-free medium. The doxorubicin-resistant phenotype of MCF-7 is not altered after 52 weeks of serial passages in drug-free medium [20].

Assay of Cytotoxic Activity

The sensitivity of doxorubicin-resistant cells to tamoxifen, megestrol acetate, doxorubicin alone, and various combinations of the drugs was determined by the SRB assay. The methodology was essentially the same as that described by Skehan *et al.* [21]. SRB binds to the amino groups of intracellular proteins in low density cell populations per well as well as at postconfluent densities for adhered cells. Cells were used in cytotoxicity studies when 90% confluence was reached in T25 flasks. Both adherent lines were harvested with 0.05% Trypsin-EDTA and counted with a Coulter counter (Coulter Electronics, model ZM). Viability was determined by trypan blue exclusion. MCF-7 and MCF-7/ADR were seeded into 96-well plates at 7×10^3 viable cells per well and left to attach to the plates for 24 hr. After 24 hr, cells were incubated for 72 hr with various concentrations of drugs in the following ranges: tamoxifen and megestrol acetate 10^{-2} to 10^2 μM plus doxorubicin 10^{-4} to 10 μM. Following a 72-hr drug exposure, the cells were fixed with 10% trichloroacetic acid for 60 min at 4°. Wells, including control, were washed five times with water and stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The wells were then washed four times with 1% acetic acid. Plates were air dried, and the dye was solubilized with 200 μL of 10 mM Tris base. The O.D. of each well was measured by spectrophotometry at 540 nm (single wavelength) with a Bio-Rad model 3550 microplate reader (Bio-Rad Laboratories, Mississauga, Canada) interfaced to an IBM compatible computer. The mean background absorbance was subtracted automatically, and mean values for each concentration (3 wells) with SEM were obtained by a microplate manager data analysis software. The IC_{50} values were calculated using sigmoidal concentration–response curve-fitting methods (Graph Pad, Prizm software) and the logit-log equation. In all cases, best fits were obtained with the coefficient of correlation R^2 ranging from 0.94 to 0.99.

ANALYSIS OF SYNERGY. Synergy was determined by the calculation $I = d_1/D_1 + d_2/D_2 + d_3/D_3$ where d is the concentration of the agent in the combination and D the concentration that would on its own be isoeffective with the

combination. I is the interaction index that is 1, <1, or >1 when the combination shows, respectively, zero interaction, synergy, or antagonism [22].

Photoaffinity Labelling

For photoaffinity labelling of P-glycoprotein, 1×10^6 cell fractions (MCF-7/ADR) were incubated with 10 nM [^{125}I]iodoarylazidoprazosin (IAAP) in the absence or the presence of 500-fold molar excess of colchicine, verapamil, vinblastine, tamoxifen, or megestrol acetate. The incubation was continued for another 30–60 min, and then was UV irradiated for 10 min at 254 nm on ice (Stratagene UV crosslinker, Stratagene, CA, U.S.A.). Following UV irradiation, cells were pelleted by centrifugation and extracted with 20 μL of 10 mM Tris–HCl, pH 8.0, containing 1% NP40 and 250 mM sucrose. The detergent-soluble proteins were fractionated on SDS–PAGE [23–25].

POLYACRYLAMIDE GEL ELECTROPHORESIS. Total cell lysate (~100 μg) from NP40-extracted cells was resolved on SDS–PAGE using the Fairbanks gel system with some modifications [23]. Briefly, the proteins were mixed with 1/5 vol. of 5× solubilization buffer I (2% SDS, 50 mM dithiothreitol, 1 mM EDTA, and 10 mM Tris–HCl, pH 8.0), and added to an equal volume of buffer II (2× buffer I, and 9 M urea). Gel slabs containing the resolved membrane proteins were fixed in 40% methanol, 10% acetic acid before drying. Dried SDS–PAGE gels were exposed to Kodak X-ray film at −70°.

FLOW CYTOMETRY. Doxorubicin accumulation in cells was tested according to Bruno and Slate [26]. Briefly, doxorubicin was added to MCF-7 and MCF-7/ADR cells at a final concentration of 2 μg/mL, along with various concentrations of megestrol acetate and tamoxifen. Treated cells were incubated at 37° for 2.5 hr for standard assays. After incubation, cells were harvested by gentle scraping, pelleted, and then washed with PBS, pH 7.0. The cells were then resuspended in PBS containing 5% FBS.

Flow cytometry was performed using a Becton Dickinson FACScan instrument on 1–3 × 10^5 cells. Forward-angle light scatter was used to define the cell population to be analysed (FL1, in arbitral units emission at 530 ± 15 nm). The LYSYS II software package was used to generate FL1 histograms and to calculate the means of the logarithmic fluorescence distribution. Data represent means of triplicates.

RESULTS

The human breast cancer cell line MCF-7/ADR was >100-fold resistant to the effects of doxorubicin as compared with MCF-7(WT) (Table 1). As previously reported [18], the IC_{50} values of tamoxifen were 10.6 μM in MCF-7/ADR and 17.5 μM in MCF-7(WT). The IC_{50} of megestrol acetate was 48.7 μM in MCF-7/ADR and 33.9 μM in MCF-7(WT). The ability of different concentrations of tamoxifen and/or megestrol acetate to partially reverse doxorubicin drug resistance was examined in MCF-7/ADR and MCF-7(WT) (Table 1). Neither tamoxifen alone nor megestrol acetate alone at 1 or 5 μM altered the cytotoxicity of doxorubicin in MCF-7/ADR cells. Tamoxifen did not sensitize the MCF-7(WT) cells to the effect of doxorubicin as previously reported [27], whereas megestrol acetate was mildly synergistic with doxorubicin. The IC_{50} of doxorubicin in MCF-7/ADR was decreased significantly in the presence of the combinations of megestrol acetate and tamoxifen (1 or 5 μM). This effect was synergistic, as indicated by the I value of <1 (Table 1). In particular, the combination of tamoxifen (5 μM) and megestrol acetate (5 μM) sensitized the MCF-7/ADR cells close to the IC_{50} concentration in WT cells (40-fold sensitization). While the combination of tamoxifen and megestrol acetate sensitized the MCF-7(WT) cells, the magnitude of the sensitization was much less (2.5-fold maximum sensitization) (Table 1).

TABLE 1. Interaction of tamoxifen, megestrol acetate, and doxorubicin in MCF-7 cells

Drug combination	MCF-7/ADR (IC_{50}, μ)M*	R^2†	I‡	MCF-7(WT) (IC_{50} μM)	R^2	I
Dox alone	1.9	0.99	–	0.012	0.98	–
Dox + tm1§	2.3	0.96	>1	0.011	0.94	0.98
Dox + tm5	1.9	0.99	>1	0.014	0.98	>1
Dox + meg1‖	2.4	0.96	>1	0.009	0.99	0.78
Dox + meg5	2.1	0.98	>1	0.006	0.99	0.65
Dox + tm1meg1	0.9	0.99	0.59	0.02	0.98	>1
Dox + tm1meg5	0.15	0.99	0.28	0.005	0.98	0.76
Dox + tm5meg1	0.32	0.99	0.66	0.005	0.99	0.73
Dox + tm5meg5	0.047	0.98	0.60	0.01	0.97	>1

* The concentration of doxorubicin necessary to produce 50% inhibition of cell growth.

† R^2 = the square of the correlation coefficient.

‡ I is the interaction index that is 1, <1, or >1 when the combination shows, respectively, zero interaction, synergy, or antagonism calulated at the IC_{50} concentration.

§ tm1 and tm5 are tamoxifen 1 μM and tamoxifen 5 μM, respectively.

‖ meg1 and meg5 are megestrol acetate 1 μM and megestrol acetate 5 μM, respectively.

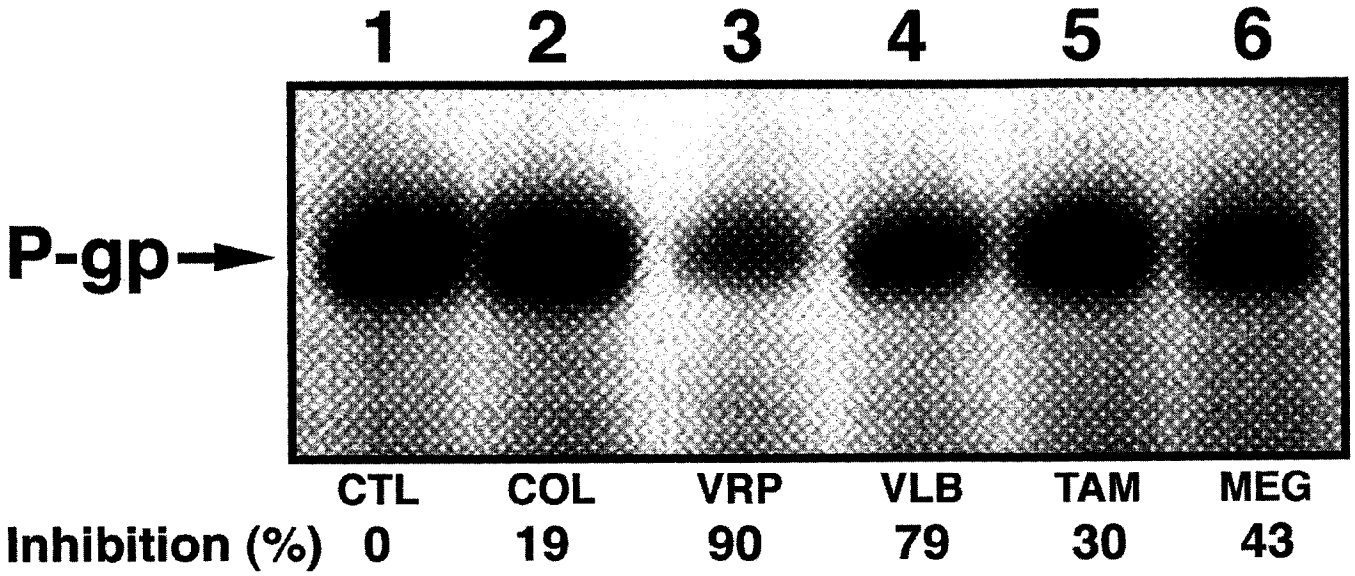

FIG. 1. Effect of drugs on photoaffinity labelling of P-glycoprotein with IAAP. MCF-7/ADR cells were photoaffinity labelled with 10 nM IAPP in the absence (lane 1) and in the presence of 500-fold molar excess of cholchicine (COL), verapamil (VRP), vinblastine (VLB), tamoxifen (TAM), or megestrol acetate (MEG) (lanes 2–6, respectively). The inhibitory effect of drugs on photoaffinity labelling of P-glycoprotein with IAAP is shown below the figure for each drug. The percent inhibition results of P-glycoprotein photoaffinity labelling with drugs were consistent and were calculated from one such experiment.

To determine if reversal of the MDR phenotype in the presence of tamoxifen or megestrol acetate is due to direct interaction of these drugs with P-glycoprotein, MCF-7/ADR cell fractions were incubated with IAAP (a P-glycoprotein specific photoactive drug [24]) in the absence and in the presence of various hydrophobic drugs, including tamoxifen or megestrol acetate. The results in Fig. 1 show the photoaffinity labelling of P-glycoprotein without drugs (lane 1) and in the presence of 500-fold molar excess of colchicine, verapamil, vinblastine, tamoxifen or megestrol acetate (lanes 2–6, respectively). These results show that the presence of verapamil or vinblastine inhibited the photoaffinity labelling of P-glycoprotein by ~90 or 79%, respectively. Similar concentrations of tamoxifen or megestrol acetate resulted in 30 or 43% inhibition of photoaffinity labelling, respectively. In contrast, colchicine, which also interacts directly with P-glycoprotein [25], inhibited P-glycoprotein photoaffinity labelling by 19%. Taken together, these results suggest that both tamoxifen and megestrol acetate inhibit P-glycoprotein photoaffinity labelling with IAAP. However, megestrol acetate was consistently better than tamoxifen.

The accumulation of doxorubicin MCF-7/ADR cells was much less than in MCF-7(WT) cells (Fig. 2). Megestrol acetate and/or tamoxifen did not increase the doxorubicin accumulation in MCF-7(WT) cells, whereas, in contrast, the combination of megestrol acetate and tamoxifen increased doxorubicin accumulation in MCF-7/ADR cells, similar to accumulation in MCF-7(WT) cells. Thus, the reversal effect of tamoxifen and megestrol acetate on the MDR phenotype of MCF-7/ADR cells is likely to be, at least partially, mediated by the competition of these drugs for the P-glycoprotein drug binding domain, resulting in increased doxorubicin accumulation.

DISCUSSION

Previous studies demonstrated that tamoxifen can partially sensitize doxorubicin-resistant cell lines at clinically obtainable concentrations [14, 15]. A recent study has demonstrated that tamoxifen at 120 mg/m^2 p.o. b.i.d. results in tamoxifen plasma concentrations of 3.1 μM and *N*-

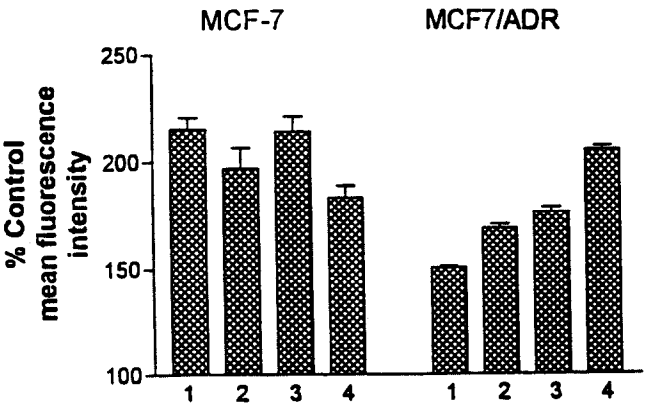

FIG. 2. Intracellular doxorubicin (Dox) accumulation in MCF-7 and MCF-7/ADR cells. Cells were exposed for 2.5 hr to 2 μg Dox/mL in the absence or presence of tamoxifen (tm) and/or megestrol acetate (meg), and the intracellular concentration of Adriamycin was measured as described in the text. Key: (1) Dox 2 μg/mL; (2) Dox 2 μg/mL + tm 5 μM; (3) Dox 2 μg/mL + meg 5 μM; and (4) Dox 2 μg/mL + tm 5 μM + meg 5 μM.

desmethyl tamoxifen of 4.9 μM [16]. The *N*-desmethyl metabolite of the tamoxifen analogue, toremifene, is approximately equivalent in its sensitizing activity to toremifene in MCF-7/ADR [15], and *N*-desmethyl tamoxifen at 4 μM decreases intrinsic multidrug resistance in human renal cell carcinoma lines in a fashion similar to 4 μM tamoxifen [28]. Thus, the concentrations of tamoxifen utilized in this study are clinically obtainable. Moreover, it has been demonstrated recently that megestrol acetate (800 mg/daily) results in a plasma concentration of 2 μM [29]. Furthermore, megestrol acetate has been given at 1600 mg daily without undue toxicity. Therefore, it should be possible to obtain clinical concentrations of 5 μM tamoxifen and probably 5 μM megestrol acetate. At these concentrations, one can sensitize the human breast cancer cell line MCF-7/ADR to the effects of doxorubicin. This effect is synergistic as indicated by the I value <1 in Table 1. In contrast, megestrol acetate alone or megestrol acetate plus tamoxifen mildly sensitized MCF-7(WT) to doxorubicin. In a previous study, megestrol acetate mildly potentiated doxorubicin cytotoxicity in the MCF-7/ADR model *in vitro* and in breast cancer sublines *in vivo* [30]. Tamoxifen, alone, in our studies failed to sensitize MCF-7/ADR cells in contrast to a previous report [15]. This may be due to differences in experimental conditions, especially the concentration of FBS (5% [15] vs 10% in our studies) since tamoxifen is extensively protein bound. Tamoxifen and its metabolites are extensively protein bound with ≤1% of the total plasma concentrations representing free drug. However, tumor concentrations of tamoxifen are substantially higher than plasma concentrations [16, 31, 32]. Thus, tamoxifen modulation of clinical doxorubicin resistance may be feasible in spite of the extensive binding to plasma proteins.

Both tamoxifen and megestrol acetate inhibited binding of IAAP to P-glycoprotein. While the combination did not further inhibit binding of IAAP (results not shown), these *in vitro* effects may not represent effects of these compounds on whole cells. Berman *et al.* [33] have demonstrated increased accumulation of doxorubicin in the presence of 10 or 50 μM tamoxifen with human MDR leukemia cells. This increased accumulation correlated with increased doxorubicin cytotoxicity. Similarly, megestrol acetate can increase doxorubicin accumulation in MDR cell lines [30]. In our investigations with tamoxifen and megestrol acetate, enhanced cytotoxicity is associated with increased doxorubicin accumulation in MCF-7/ADR cells, which is likely mediated by competition of tamoxifen and megestrol acetate with doxorubicin for the P-glycoprotein binding domain.

In summary, clinically obtainable concentrations of tamoxifen and megestrol acetate can synergistically sensitize MCF-7/ADR to doxorubicin and mildly sensitize MCF-7(WT) cells. Studies in *in vivo* models should determine the potential therapeutic benefit of this combination.

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References

1. Falkson G, Gelman RS, Tormey DC, Cummings FJ, Carbone PP and Falkson HC, The Eastern Cooperative Oncology Group experience with cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) in patients with metastatic breast cancer. *Cancer* **56:** 219–224, 1985.
2. Muss HB, White DR, Richards F, Cooper RM, Stuart JJ, Jackson DV, Rhyne L and Spurr CL. Doxorubicin versus methotrexate in five-drug combination chemotherapy for advanced breast cancer. *Cancer* **42:** 2141–2148, 1978.
3. Nemoto T, Horton J, Simon R, Dao TL, Rosner D, Cunningham T, Sponzo R and Synderman M, Comparison of four combination chemotherapy programs in metastatic breast cancer. *Cancer* **49:** 1988–1993, 1982.
4. Vogel CL, Smalley RV, Raney M, Krauss S, Carpenter J, Velez-Garcia E, Fishkin E, Raab S, Moore MR and Stagg M, Randomized trial of cyclophosphamide, doxorubicin and 5-fluororacil alone or alternating with a "cycle active" noncross-resistant combination in women with visceral metastatic breast cancer: A Southeastern Cancer Society group project. *J Clin Oncol* **2:** 643–651, 1984.
5. Tormey DC, Gelman R, Band PR, Sears M, Rosenthal SN, DeWys W, Perlia C and Rice MA, Comparison of induction chemotherapies for metastatic breast cancer. *Cancer* **50:** 1235–1244, 1982.
6. Hoogstraten MD, George SL, Samal B, Rivkin SE, Costanzi JJ, Bonnet JD, Thipen T and Braine H, Combination chemotherapy and doxorubicin in patients with advanced breast cancer. *Cancer* **38:** 13–20, 1976.
7. Carmo-Pereira J, Costa FL and Henriques E, Chemotherapy of advanced breast cancer—A randomized trial of vincristine, doxorubicin and cyclophosphamide (VAC) versus cyclophosphamide, methotrexate, 5-fluorouracil and prednisone (CMFP). *Cancer* **48:** 1517–1521, 1981.
8. Perez DJ, Harvey VJ, Robinson BA, Atkinson CH, Dady PJ, Kirk AR, Evans BD and Chapman PJ, A randomized comparison of single-agent doxorubicin and epirubicin as first-line cytotoxic therapy in advanced breast cancer. *J Clin Oncol* **9:** 2148–2152, 1991.
9. Moscow JA and Cowan KH, Multidrug resistance. *J Natl Cancer Inst* **80:** 14–20, 1988.
10. Tsuruo T, Iida H, Tsukagoshi S and Sakurai Y, Increased accumulation of vincristine and doxorubicin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. *Cancer Res* **42:** 4730–4733, 1982.
11. Slater LM, Sweet P, Stupecky M, Wetzel MW and Gupta S, Cyclosporine A corrects daunorubicin resistance in Ehrlich ascites carcinoma. *Br J Cancer* **54:** 235–238, 1986.
12. Ramu A, Fuks Z, Gatt S and Glaubiger D, Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by perhexiline maleate. *Cancer Res* **44:** 144–148, 1984.
13. Cornwell MM, Gottesman MM and Pastan I, Increased vinblastine binding to membrane vesicles from multidrug resistant KB cells. *J Biol Chem* **261:** 7921–7928, 1986.
14. Ramu A, Glaubiger D and Fuks Z, Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. *Cancer Res* **44:** 4392–4395, 1984.
15. DeGregorio MW, Ford JM, Benz CC and Wiebe VJ, Toremifene: Pharmacologic and pharmacokinetic basis of reversing multidrug resistance. *J Clin Oncol* **7:** 1359–1364, 1989.
16. Trump DL, Smith SC, Ellis PG, Rogers MP, Schold MP, Winer EP, Panella TJ, Jordan VC and Fine RL, High dose oral tamoxifen, a potential multidrug-resistance-reversal agent: A phase I trial in combination with vinblastine. *J Natl Cancer Inst* **84:** 1811–1816, 1992.

17. Yang C-PH, Depinho SG, Greenberger LM, Arceci RJ and Horwitz SB, Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of the gravid uterus. *J Biol Chem* **264:** 782–789, 1989.
18. Batist G, Tulpule A, Sinha BK, Katki AG, Myers CE and Cowan KH, Overexpression of a novel anionic glutathione transferase in multidrug resistant human breast cancer cells. *J Biol Chem* **261:** 15544–15549, 1986.
19. Kramer R, Zakher J and Kim G, Role of the glutathione redox cycle in acquired and *de novo* multidrug resistance. *Science* **241:** 694–697, 1988.
20. Vickers P, Dickson R, Shoemaker R and Cowan K, A multidrug-resistant MCF-7 human breast cancer cell line which exhibits cross-resistance to anti-estrogens and hormone-independent tumor growth. *Mol Endocrinol* **2:** 886–892, 1988.
21. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR, New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* **82:** 1107–1112, 1990.
22. Berenbaum MC, Letter to the Editor. *Cancer Res* **52:** 4558–4559, 1992.
23. Fairbanks G, Steck TL and Wallach DFH, Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry* **10:** 2606–2617, 1971.
24. Greenberger LM, Major photoaffinity drug labeling sites for iodoaryl azidoprazosin in P-glycoprotein are within, or immediately C-terminal to, transmembrane domains 6 and 12. *J Biol Chem* **268:** 11417–11425, 1993.
25. Safa AR, Mehta ND and Agresti M, Photoaffinity labelling of P-glycoprotein in multidrug resistant cells with photoreactive analogs of colchicine. *Biochem Biophys Res Commun* **162:** 1402–1408, 1989.
26. Bruno NA and Slate DL, Effect of exposure to calcium entry blockers on doxorubicin accumulation and cytotoxicity in multidrug-resistant cells. *J Natl Cancer Inst* **82:** 419–424, 1990.
27. Woods KE, Randolph JK and Gewirtz DA, Antagonism between tamoxifen and doxorubicin in the MCF-7 human breast tumor cell line. *Biochem Pharmacol* **47:** 1449–1451, 1994.
28. Fine RL, Sachs CW, Albers M and Williams A, Tamoxifen potentiates the cytotoxicity of vinblastine by increasing intracellular drug accumulation. *Proc Am Assoc Cancer Res* **32:** 375, 1991.
29. Wang L, Yang C-PH, Trial P, Horwitz SB and Casazza AM, Reversal of the multidrug resistance (MDR) phenotype with megesterol acetate (MA). *Proc Am Assoc Cancer Res* **32:** 377, 1991.
30. Wang L, Yang C-PH, Horwitz SB, Trial PA and Casazza AM, Reversal of the human multidrug-resistance phenotype with megestrol acetate. *Cancer Chemother Pharmacol* **34:** 96–102, 1994.
31. Daniel P, Gaskell SJ, Bishop H, Campbell C and Nicholson R, Determination of tamoxifen and biologically active metabolites in human breast tumors and plasma. *Eur J Cancer Clin Oncol* **17:** 1183–1189, 1981.
32. DeGregorio MW, Wilbur BJ, Coronado E and Osborne CK, Serum tamoxifen concentrations in the athymic nude mouse after three methods of administration. *Cancer Chemother Pharmacol* **20:** 316–318, 1987.
33. Berman E, Adams M, Duigou-Osterndorf R, Godfrey L, Clarkson B and Andreeff M, Effect of tamoxifen on cell lines displaying the multidrug-resistant phenotype. *Blood* **77:** 818–825, 1991.