Cancer Chemother Pharmacol (1995) 35: 457–463

ORIGINAL ARTICLE

Susan E. Bates · Beverly Meadows · Barry R. Goldspiel
Andrea Denicoff · Tung Ba Le · Eben Tucker
Seth M. Steinberg · Lori J. Elwood

A pilot study of amiodarone with infusional doxorubicin or vinblastine in refractory breast cancer

Received: 19 January 1994 / Accepted: 5 August 1994

Abstract Increasing evidence suggests that P-glycoprotein (Pgp) expression can mediate drug resistance in refractory breast cancer. We studied 33 patients with refractory breast cancer enrolled in a pilot study of oral amiodarone as a Pgp antagonist given in combination with infusional doxorubicin or vinblastine. Whenever possible, tumors were biopsied and Pgp expression was assayed. Patients received either 60 mg/m^2 doxorubicin over 96 h or 8.5 mg/m^2 vinblastine over 120 h by continuous intravenous infusion. Beginning with the second cycle of chemotherapy, 600–800 mg amiodarone was given orally each day. Patients who experienced toxicity due to amiodarone but were responding to chemotherapy were placed on quinidine. Partial responses were observed in 9 of 33 patients on study and were sometimes observed after the first cycle of chemotherapy, before amiodarone was given, suggesting that some patients may have responded to treatment because of the infusional schedule. Toxicities were primarily the known side effects of the antineoplastic agents and of amiodarone. The major amiodarone toxicity was gastrointestinal, with nausea, vomiting, anorexia, or diarrhea being noted in 21 patients. Biopsy samples were obtained from 29 patients and in 21 cases, viable tumor tissue was present and the results were interpretable. Of the 21 samples, 9 had Pgp expression as determined by immunohistochemical staining; 12 were considered negative. The presence of Pgp expression was associated with an acceleration of the time to treatment failure. Whereas normal-tissue toxicities related to the combination of a Pgp antagonist with chemotherapy were not observed, amiodarone was associated with too many untoward effects to be utilized as a drug resistance-reversing agent.

Key words Amiodarone · P-glycoprotein · Breast cancer

S. E. Bates (✉)
Building 10, Room 12N226, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, USA

S. E. Bates · B. Meadows · A. Denicoff · T. B. Le
Medicine Branch, National Cancer Institute, 9000 Rockville, Pike, Bethesda, MD 20892, USA

B. R. Goldspiel
Pharmacy Department, Warren G. Magnuson Clinical Center, Bethesda, MD 20892, USA

E. Tucker
Cardiology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD 20892, USA

S. M. Steinberg
Biostatistics and Data Management Section, National Cancer Institute, Bethesda, MD 20892, USA

L. J. Elwood
Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20814, USA

Introduction

Treatment of metastatic breast cancer with cytotoxic therapy is limited by relapse and treatment failure. A review of clinical trials calculated a 16.6-month median survival for women with metastatic breast cancer treated with conventional chemotherapy [6]. The search for mechanisms underlying drug resistance in breast cancer has led to evaluation of glutathione redox pathways and of oncogene and P-glycoprotein (Pgp) expression [23]. In laboratory models, the overexpression of Pgp has been shown to mediate multidrug resistance through active drug efflux, thereby reducing the intracellular concentration of multiple agents [25]. The agents effluxed are principally natural products, including vincristine, vinblastine, actinomycin D, doxorubicin, paclitaxel, and mitoxantrone. Increasing evidence supports a role for Pgp in clinical drug resistance as well. The expression of Pgp or the encoding gene *mdr*-1 has been correlated with diminished survival in childhood sarcoma, neuroblastoma, and adult acute leukemia [2–4, 20].

Evidence that Pgp mediates clinical drug resistance in breast cancer is also accumulating, with various investigators noting increased Pgp expression in patients previously treated for breast cancer [29, 31, 32]. Using a variety of

methodologies, reports of Pgp expression have ranged from 0 to 85% in the samples studied [10, 21, 29, 31, 32]. The variance in expression is most likely due to the methods used, including those that evaluate the entire tumor population (RNA analysis by slot or Northern blotting or protein analysis by Western blotting) and immunohistochemical techniques [10, 21, 29, 31, 32].

In laboratory models, numerous agents have been shown to inhibit active drug efflux, including verapamil, quinidine, phenothiazines, amiodarone, and cyclosporine A. This finding has led to various clinical trials testing whether anticancer therapy can be improved by adding a Pgp antagonist to cytotoxic therapy. The choice of a Pgp antagonist has largely centered on availability, with agents that are in clinical use for other indications, such as verapamil or cyclosporine, being the first tested [13, 22, 26, 33]. However, concentrations of verapamil achieved at maximal tolerated doses are well below those needed for in vitro reversal studies [22, 24]. Cyclosporine can be given in doses that achieve serum levels comparable with those used in vitro, but it demonstrates significant toxicity [18, 33, 39]. Amiodarone is an effective antagonist in vitro at concentrations that can be found in the serum of patients receiving the drug for cardiac indications [5, 8, 9, 37]. Amiodarone doses ranging from 1 to 3 μg/ml are as effective as 10 μ*M* verapamil in antagonizing Pgp in vitro.

In the present study, patients with refractory breast cancer were treated with a combination of amiodarone and doxorubicin (or vinblastine if the patients' cumulative doxorubicin dose exceeded 240 mg/m^2). Both doxorubicin and vinblastine were given by continuous infusion in doses previously noted to be effective in breast cancer [7, 17]. Biopsy samples were obtained from as many patients as possible, and the expression of Pgp was assessed by immunohistochemistry. The study was designed primarily to test the feasibility of using amiodarone as a Pgp antagonist in breast cancer and also to assess the expression of Pgp in the population of patients enrolled.

Patients and methods

Patient selection

Patients entering on study had recurrent breast cancer. Prior therapies included chemotherapy (with and without anthracyclines), surgery, hormonal therapy, irradiation, and autologous bone marrow transplantation (two patients). Patients had a life expectancy of greater than 12 weeks along with a Karnofsky performance status of greater than 40%. Radiation therapy must have been completed 6 weeks prior to their entering the study. All patients gave informed consent as dictated by Institutional Review Board policies.

Treatment program

All patients had a temporary triple-lumen or permanent central-venous access device placed for the administration of doxorubicin or vinblastine. Doxorubicin (60 mg/m^2) was dissolved in 0.9% Sodium Chloride, USP, and was delivered by continuous intravenous infusion over 96 h using a portable infusion pump (Pharmacia Deltec CADD-1, Pharmacia Delta, Inc., St. Paul, Minn.). Treatment was changed from doxorubicin to vinblastine if the ejection fraction decreased while on study or if the cumulative doxorubicin dose exceeded 525 mg/m^2. Vinblastine was initially given to patients with a cumulative doxorubicin dose of greater than 240 mg/m^2 or an ejection fraction of $<45\%$. The total vinblastine dose (8.5 mg/m^2) was divided in half and diluted to a total volume of 100 ml with 0.9% Sodium Chloride for Injection, USP, and was delivered as a continuous intravenous infusion over 120 h using the same portable pump. Two reservoirs, each infused over 60 h, were required to complete the 120-h infusion. The first cycle of chemotherapy was given without concurrent amiodarone.

Amiodarone loading at 1000 mg/day began after completion of the first chemotherapy infusion and continued for 2 weeks. A daily dose of 600–800 mg amiodarone was given throughout subsequent chemotherapy cycles. If a patient developed toxicity or intolerable side effects due to amiodarone, quinidine sulfate at 200 mg q.i.d. could be substituted as the Pgp antagonist and titrated to a serum level of 5 μg/ml. Quinidine was given for 2 days prior to and concurrently with the chemotherapy infusion. Patients were monitored in the intensive care unit while receiving quinidine due to the potential for cardiac sequelae from the synergistic activity of amiodarone and quinidine given in close proximity. Patients were continued on therapy until documentation of progressive disease. Standard criteria were applied for determination of response.

Amiodarone and quinidine assays

Quinidine was assayed at the Warren G. Magnuson Clinical Center laboratory by a fluorescence polarization immunoassay (TDx system, Abbott Laboratories, Diagnostics Division, Abbott Park, Ill.). Amiodarone and desethylamiodarone were analyzed by a high-performance liquid chromatography (HPLC) assay at Smith-Kline-Beecham (Philadelphia, Pa.).

Immunohistochemical staining

Patients' samples were obtained by various means from a variety of sources. These included 6-mm punch biopsies of skin lesions; excisional, trucut, or needle-aspiration biopsies of breast, pulmonary, or hepatic metastases; thoracentesis; and paracentesis. Cell pellets obtained from suspensions and tumor biopsies were embedded in OCT over a dry-ice/methanol bath. For confirmation that the biopsy samples contained tumor tissue, hematoxylin-and-eosin-stained frozen sections were evaluated in addition to the immunohistochemical stains.

Frozen sections (8 μm) were thawed, dried, fixed for 10 min in 3.7% formaldehyde, and then washed in phosphate-buffered saline. Subsequently, slides were hybridized as previously described for 1 h at 23 °C with either MRK-16 antibody (10 μg/ml), which is specific for Pgp, or IgG δ2a (10 μg/ml; Coulter Immunology, Hialeah, Fla.) [1, 11, 34, 35]. A peroxidase-conjugated anti-mouse antibody (Jackson Immunoresearch Laboratories, Westgrove, Pa.) was used to detect MRK-16 binding. Each tumor was examined in duplicate in a minimum of three separate experiments.

Results

Patients' characteristics

Patients ranged in age from 31 to 74 years. A total of 27 patients had received prior anthracycline therapy; 14 had received a cumulative dose of over 240 mg/m^2 and were placed on vinblastine. Patients had received a mean of 2.2 (range, 0–5) different chemotherapy regimens for metastatic disease prior to entry on this study and a mean of 3.1 (range, 0–6) different regimens including biological and hormonal therapy.

Responses

Disease response was evaluable in 29 of the 33 patients on study. Four patients were inevaluable due to early withdrawal or death. No patient had a complete remission. Nine patients had a partial response, three had a minimal response, nine had stable disease, and eight had progressive disease. Tumor shrinkage had to persist for 1 month or more to be considered a response. Patients were on study for a median of 3 (range, 1–5) cycles. Favorable responses to treatment were often of short duration, sometimes lasting only one cycle. Many of the patients had pulmonary or chest-wall metastases, both of which could be evaluated after the first cycle of therapy without amiodarone. Several of the patients had tumor shrinkage following that first cycle. This suggested that tumors in many patients were not refractory to the chemotherapy alone.

A slight apparent difference in the response rate was noted between patients receiving doxorubicin (31%) and those receiving vinblastine (21%). This was not statistically significant ($P \geq 0.5$, Fisher's exact test) and was probably related to the amount of prior therapy, since patients enrolled on vinblastine had received more than 240 mg/m^2 doxorubicin.

Amiodarone levels

The study design targeted amiodarone serum levels near 2 μg/ml. Most patients required 800 mg/day and not all achieved target levels. The mean (± SD) level of amiodarone was 1.67 ± 0.79 μg/ml, and that of the metabolite desethylamiodarone was 1.11 ± 0.47 μg/ml. Amiodarone levels in patients ranged from 0.3 to 3.45 μg/ml, and desethylamiodarone levels varied from 0.3 to 3.0 μg/ml. No correlation was observed between the serum level of amiodarone and the response to therapy (the amiodarone level was 1.71 ± 0.80 μg/ml in patients with a partial response and 1.60 ± 0.80 μg/ml in nonresponders).

Toxicities

Three types of toxicity were evaluated in this study: those due to the antineoplastic agent alone, those due to the antagonists alone, and those due to the combination of the two. The major toxicities of chemotherapy were neutropenia and cardiac complications. Neutropenia (absolute neutrophil count, less than 500/mm^3) occurred in a majority of the patients, but only five required hospital admission for fever. Four patients were changed from doxorubicin to vinblastine because of a cumulative doxorubicin dose of 610 mg/m^2 (two patients) or a declining ejection fraction (two patients).

Cardiac complications due to both amiodarone and quinidine were observed. Among 31 patients receiving amiodarone, 8 developed a first-degree atrioventricular (AV) block, with 1 patient demonstrating a junctional rhythm. One patient developed a second-degree AV block that was asymptomatic. All cases of heart block resolved upon discontinuance of amiodarone. Among the five patients receiving quinidine, prolonged QTc intervals were observed that returned to normal upon quinidine discontinuance.

Amiodarone has known pulmonary, ocular, thyroid, and gastrointestinal toxicities [9]. No patient had a decrease in pulmonary function that could be attributed solely to amiodarone. However, lung metastases and pleural effusions were so common that amiodarone-related lung toxicity may have been difficult to discern. A deterioration in pulmonary status was always associated with progression of disease and/or recurrent pleural effusions. Ocular keratopathy due to amiodarone is characterized as pigmented microdeposits on the corneal epithelium and manifests as a halo effect in the visual field [9, 38]. Amiodarone was discontinued in three patients documented by ophthalmologic examination to have ocular keratopathy. Patients were also monitored for changes in thyroid and adrenal function. Three patients whose T4 levels declined were placed on thyroid supplementation and amiodarone was continued. No clinical evidence of adrenal toxicity was found during the study. Anorexia, nausea, vomiting, or diarrhea was frequently observed (in 21 of 33 patients) and attributed to amiodarone; however, only 1 patient required discontinuation of amiodarone because of nausea. No hyperbilirubinemia related to amiodarone was observed. One patient with multiple severe liver metastases was suspected of having concurrent amiodarone-related liver toxicity. Five patients were treated with quinidine because of amiodarone toxicity.

The toxicities described above are those expected following single-agent administration of these substances. The third form of potential toxicity encountered in combining chemotherapy with a Pgp antagonist is that due to sensitization of normal tissues where Pgp is expressed. The only potential toxicity that could be attributed to Pgp antagonism in normal tissue was neurotoxicity. Five patients had weakness, paresthesia, dysesthesia, or muscle cramps in the lower extremities: two patients receiving quinidine and three patients receiving amiodarone. Four of the five patients who complained of these neurological symptoms were receiving vinblastine.

Amiodarone, desethylamiodarone, and total amiodarone plus desethylamiodarone levels were analyzed for correlations with the presence of ocular, thyroid, gastrointestinal, and neurological toxicities. No statistically significant difference was found by the Wilcoxon rank-sum test; however, a trend toward higher total amiodarone plus desethylamiodarone levels was observed in the three patients with neurotoxicity (4.36 ± 2.04 versus 2.36 ± 1.08 μg/ml, $P = 0.09$).

P-glycoprotein expression

Tumor biopsies were obtained from 29 patients. Viable tumor was found in only 21 of the biopsies, whereas the rest demonstrated necrosis, fibrosis, or normal tissue. Biopsies taken under computerized tomographic (CT) guidance from known metastatic sites occasionally contained

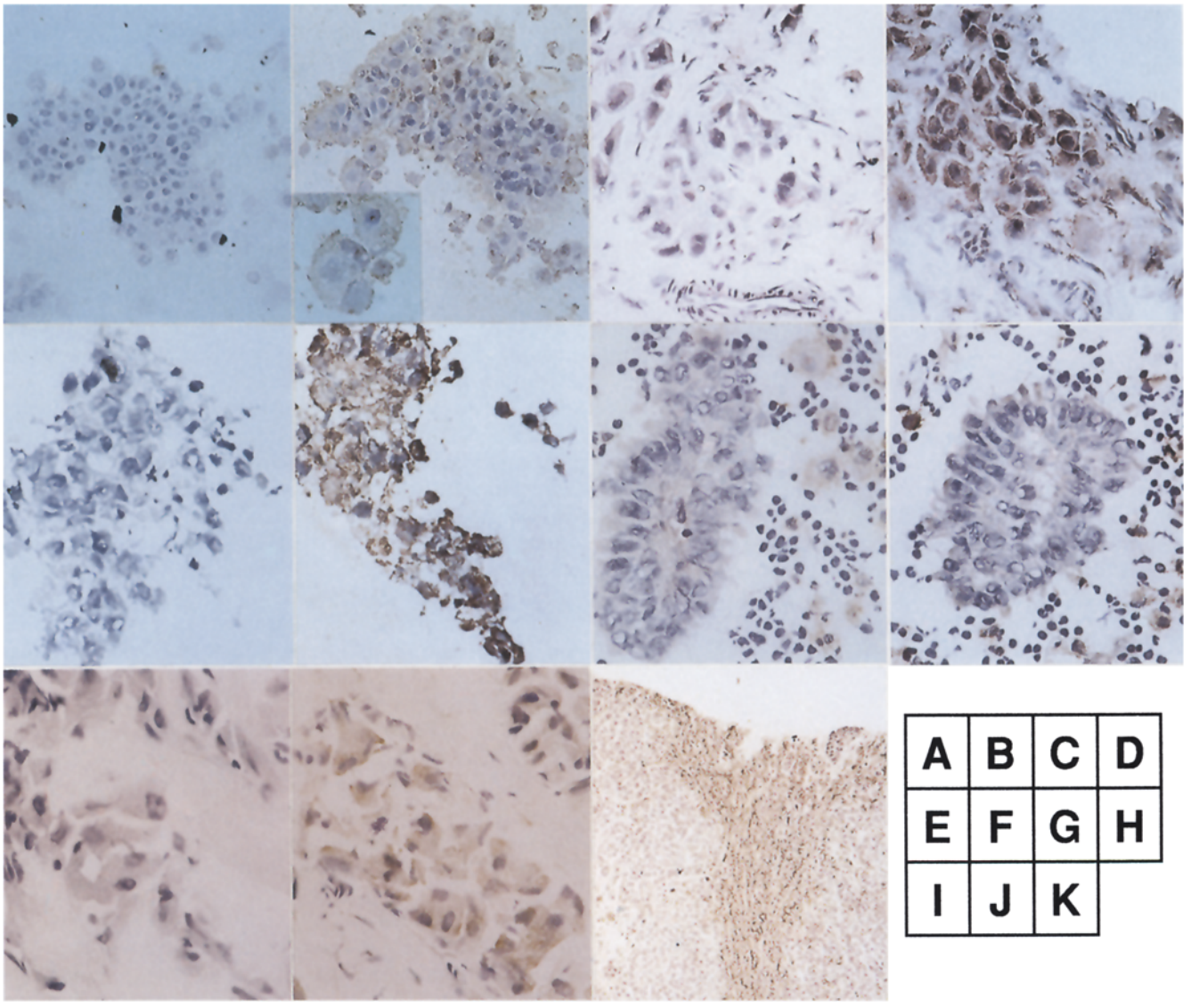

Fig. 1 A–K Immunohistochemical analysis of Pgp expression. Tumor biopsies were stained using MRK-16 to detect Pgp or IgGg2a as a control antibody with peroxidase detection. Representative positive and negative samples are shown. **A, C, E, G, I** IgGg2a control stains for nonspecific staining. **B, D, F, H, J, K** MRK-16 staining. **A, B** Positive staining for Pgp in tumor cells in pleural effusion. **C, D** Positive staining for Pgp in tumor cells only in a breast biopsy. **E, F** Positive staining for Pgp in tumor cells from lung aspirate. **G, H** Absence of staining for Pgp in tumor cells in pleural effusion. **I, J** Positive staining for Pgp in tumor cells only in a breast biopsy. **K** Typical bile canalicular staining in normal liver and absence of staining in the tumor sample

only normal tissue. Immunohistochemical staining was performed with MRK-16; representative samples are shown in Fig. 1. Expression of P-glycoprotein (Pgp) was observed in 9 of 21 (43%) of the tumor samples (Table 1). Samples were considered positive if staining was observed in 20% of the identifiable tumor cells. Staining for Pgp was confirmed by a minimum of three separate experiments.

Clinical correlations

Associations between Pgp expression and response, time to treatment failure, and prior doxorubicin therapy were evaluated by univariate methods. Pgp expression did not correlate with response or prior therapy. However, as shown in Fig. 2, the presence of Pgp as assayed by immunohistochemical staining was associated with a shorter time to treatment failure by the Kaplan-Meier method (median time, 3.1 versus 5.6 months; $P = 0.0064$, Mantel-Haenszel method) [14, 19]. One of the nine patients with positive Pgp expression was excluded from this analysis because she died before receiving two cycles of therapy and was thus inevaluable.

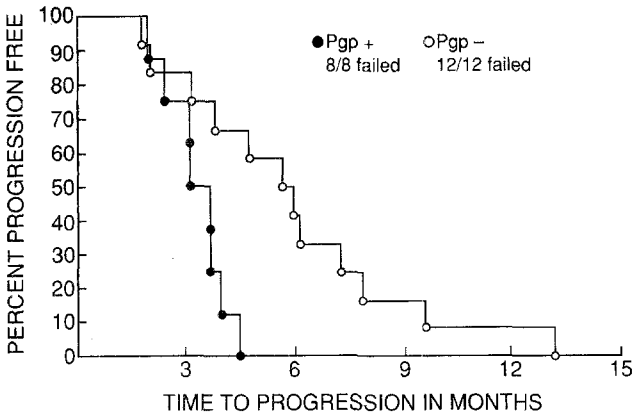

Fig. 2 Kaplan-Meier curve demonstrating the relationship between the time to disease progression and Pgp expression. An immunohistochemical assay was performed on tumor biopsies obtained from patients at the time of study entry

Table 1 Pgp expression in biopsy samples[a]

Response	Number of patients[a]	Pgp expression		No tumor[b]
		Pgp+	Pgp−	
Partial	9	3	6	0
Stable[c]	12	3	3	3
Progression	8	2	3	2
Not evaluable	4	1	0	3
Totals	33	9	12	8

[a] Biopsies were not obtained from 4 patients: 3 with stable disease and 1 with disease progression

[b] Although tumor samples were obtained from known tumor sites, viable tumor cells were not seen in frozen sections. Either the tumor was necrotic or only normal cells were observed

[c] Stable disease includes patients with minimal responses

Discussion

Investigators have addressed the importance of Pgp in cancer treatment by evaluating the incidence and level of Pgp expression in clinical samples and by attempting reversal of Pgp-mediated resistance. The present study treated patients with advanced breast cancer with amiodarone as a Pgp antagonist combined with either doxorubicin or vinblastine. Amiodarone was given daily; chemotherapy was given in 3- to 4-week cycles by continuous intravenous infusion. In all, 9 partial responses were observed among 33 patients, but it is not possible to discern the contribution of the amiodarone, which resulted in multiple adverse effects itself. Pgp was expressed in 43% of the tumor specimens and correlated with a shortened time to treatment failure.

Clinical studies defining a role for multidrug resistance by evaluation of results obtained with reversal agents are in their infancy. Early trials, like the present one, used antagonists that were commercially available for other indications [13, 22, 24, 26, 39]. These antagonists usually cause toxicity at the levels needed for full reversal of drug resistance in vitro [18, 24, 26, 39]. The majority of these trials have been disappointing, producing few and short-lived responses. However, actual Pgp antagonism and proof of increased drug accumulation in tumor tissue has not yet been documented in any clinical study. Newer, more potent antagonists may improve the clinical outcome.

The continuous-infusion schedule was chosen in part because of its safety in patients previously treated with doxorubicin [12, 17]. Subsequent laboratory studies have indicated that prolonged exposure of cells to doxorubicin, vincristine, or paclitaxel can reduce the relative resistance of cells bearing Pgp ([15]; unpublished observations). Thus, prolonging the exposure duration by giving doxorubicin or vinblastine by continuous infusion may have resulted in some of the responses observed. The trial design in the present study did not require proof that patients were refractory to the therapy before the addition of a reversing agent. Thus, the responses observed cannot be directly attributed to amiodarone.

It has been argued that amiodarone and other protein-bound agents may be poor antagonists in vivo. Although amiodarone is highly protein-bound, pharmacokinetics studies have shown rapid distribution to normal tissues after intravenous injection [28]. At 4–16 h after a single i. v. dose in rats, amiodarone is found in the adipose tissue, kidney, liver, heart, skeletal muscle, and brain at concentrations that are 1–2 logs higher than those found in blood [28]. After chronic dosing, significant accumulation occurs in these tissues as well [16]. Pharmacokinetics studies in normal volunteers suggest that extensive tissue distribution also occurs in humans [27]. Although the levels achieved in tumors are unknown, these studies demonstrate that protein binding does not interfere with tissue distribution. Whereas the 2-μg/ml target concentration of amiodarone was achieved in only 7 of 29 patients in the present study, there is evidence that the metabolite desethylamiodarone is also capable of reversing drug resistance [37].

Toxicities observed in patients on this study were primarily known toxicities of the individual drugs. Toxicities due to doxorubicin or vinblastine did not appear to be excessive for this patient population. Toxicities specific to amiodarone or quinidine were noted, and those of amiodarone included cardiac conduction abnormalities, visual problems, changes in thyroid function tests, and gastrointestinal complaints. Neurotoxicity, observed in five patients, was suspected to be an enhanced normal-tissue toxicity due to the combination of vinblastine with a Pgp antagonist. Similar neurological toxicities were observed in recently reported trials combining cyclosporine A or tamoxifen with vinblastine [30, 36]. Increased myelosuppression or hyperbilirubinemia, as reported in reversal studies utilizing cyclosporine A [18, 39], was not observed in this study.

Immunohistochemistry detected Pgp expression in 9 of 21 evaluable biopsy samples from patients on this study. Biopsy samples were obtained from 29 metastatic sites in the liver, skin, breast, or lung, tissues that have differing levels of intrinsic Pgp expression. Immunohistochemistry has the advantage of determining the level of Pgp expression

in the malignant cells, regardless of the expression in the surrounding normal tissue. Only 21 of the 29 samples contained viable tumor, illustrating the importance of histologically verifying tumor involvement in any method of Pgp analysis.

There was no correlation of Pgp expression with response, but expression was associated with a shorter time to treatment failure ($P = 0.0064$, Mantel-Haenszel method). Regardless of the response observed, patients whose tumors were positive for Pgp developed progression of disease sooner than patients whose tumors were negative. This finding is consistent with reports in other diseases [2–4, 20]. The presence of a correlation with treatment failure in our study also suggests that Pgp antagonism by amiodarone was not effective.

In summary, this study demonstrated Pgp expression in patients with refractory breast cancer. Administration of amiodarone as a Pgp antagonist in combination with infusional chemotherapy resulted in minimal normal-tissue toxicity but in significant toxicities known for amiodarone. The study design does not permit conclusions to be drawn about the effectiveness of amiodarone as a Pgp antagonist because resistance to the antineoplastic regimen alone was not confirmed and proof that amiodarone was effectively blocking Pgp was not obtained. Increased experience with Pgp-reversal trials has shown that this type of trial design cannot provide a definitive answer as to whether Pgp modulation can improve cancer treatment. Instead, trials with a crossover design are needed in which the patient is treated with chemotherapy alone prior to the addition of an antagonist when disease progression occurs. Ultimate proof that Pgp antagonists can improve cancer therapy awaits the performance of randomized trials in previously untreated patients.

Acknowledgements The authors would like to thank Drs. Kenneth Cowan, Antonio Fojo, and Robert Wittes for advice and encouragement, Anika Lynch for help with the immunochemical assays, and Ricardo Dreyfuss for high-quality photography.

References

1. Bates SE, Shieh CY, Tsokos M (1991) Expression of *mdr*-1/P-glycoprotein in human neuroblastoma. Am J Pathol 139: 305–315
2. Campos L, Guyotat D, Archimbaud E, Calmard-Oriol P, Tsuruo T, Troncy J, Treille D, Fiere D (1992) Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. Blood 79: 473–476
3. Chan HSL, Thorner PS, Haddad G, Ling V (1990) Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. J Clin Oncol 50: 2818–2822
4. Chan HSL, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V (1991) P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 325: 1608–1614
5. Chauffert B, Rey D, Coudert B, Dumas M, Martin F (1987) Amiodarone is more efficient than verapamil in reversing resistance to anthracyclines in tumour cells. Br J Cancer 56: 119–122
6. Eddy DM (1992) High-dose chemotherapy with autologous bone marrow transplantation for the treatment of metastatic breast cancer. J Clin Oncol 10: 657–670
7. Fraschini G, Yap HY, Hortobagyi GN, Buzdar A, Blumenschein G (1985) Five-day continuous-infusion vinblastine in the treatment of breast cancer. Cancer 56: 225–229
8. Genne P, Coudert B, Pelletier H, Girardot C, Martin F, Chauffert B (1989) Serum concentrations of amiodarone required for an in vivo modulation of anthracycline resistance. Anticancer Res 9: 1655–1660
9. Gill J, Heel RC, Fitton A (1992) Amiodarone: an overview of its pharmacological properties, and review of its therapeutic use in cardiac arrhythmias. Drugs 43: 69–110
10. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazder A, Pirker R, Green A, Crist W, Brodeur GM, Lieber M, Cossman J, Gottesman MM, Pastan I (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81: 116–124
11. Herzog CE, Trepel JB, Mickley LA, Bates SE, Fojo AT (1992) Various methods of analysis of *mdr-1*/P-glycoprotein in human colon cancer cell lines. J Natl Cancer Inst 84: 711–716
12. Hortobagyi GN, Frye D, Buzdar AU, Ewer MS, Fraschini G, Hug V, Ames F, Montague E, Carrasco CH, Mackay B, Benjamin RS (1989) Decreased cardiac toxicity of doxorubicin administered by continuous intravenous infusion in combination chemotherapy for metastatic breast carcinoma. Cancer 63: 37–45
13. Isonishi S, Kirmani S, Kim S, Plaxe SC, Braly PS, McClay EF, Howell SB (1991) Phase I and pharmacokinetic trial of intraperitoneal etoposide in combination with the multidrug-resistance-modulating agent dipyridamole. J Natl Cancer Inst 83: 621–626
14. Kaplan E, Meier P (1958) Non-parametric estimation from incomplete observations. J Am Stat Assoc 53: 457–481
15. Lai G-M, Chen Y-N, Mickley LA, Fojo AT, Bates SE (1991) P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma cell lines. Int J Cancer 49: 696–703
16. Latini R, Bizzi A, Cini M, Veneroni E, Marchi S, Riva E (1986) Amiodarone and desethylamiodarone tissue uptake in rats chronically treated with amiodarone is non-linear with the dose. J Pharm Pharmacol 39: 426–431
17. Legha SS, Benjamin RS, Mackay B, Yap HY, Wallace S, Ewer M, Blumenschein GR, Freireich EJ (1982) Adriamycin therapy by continuous intravenous infusion in patients with metastatic breast cancer. Cancer 49: 1762–1766
18. Lum BL, Kaubisch S, Yahanda AM, Adler KM, Jew L, Ehsan MN, Brophy NA, Halsey J, Gosland MP, Sikic BI (1992) Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. J Clin Oncol 10: 1635–1642
19. Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50: 163–170
20. Marie J-P, Zittoun R, Sikic BI (1991) Multidrug resistance (mdr1) gene expression in adult acute leukemias: correlations with treatment outcome and in vitro drug sensitivity. Blood 78: 586–592
21. Merkel DE, Fuqua SAW, Tandon AK, Hill SM, Buzdar AU, McGuire WL (1989) Electrophoretic analysis of 248 clinical breast cancer specimens for P-glycoprotein overexpression or gene amplification. J Clin Oncol 7: 1129–1136
22. Miller TP, Grogan TM, Dalton WS, Spier CM, Scheper RJ, Salmon SE (1991) P-glycoprotein expression in malignant lymphomas and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. J Clin Oncol 9: 17–24
23. Morrow CS, Cowan KH (1988) Mechanisms and clinical significance of multidrug resistance. Oncology 2: 55–68
24. Ozols RF, Cunnion RE, Klecker RW, Hamilton TC, Ostchega Y, Parrillo JE, Young RC (1987) Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol 5: 641–647
25. Pastan I, Gottesman M (1987) Multiple-drug resistance in human cancer. N Engl J Med 316: 1388–1393
26. Philip PA, Joel S, Monkman SC, Dolega-Ossowski E, Tonkin K, Carmichael J, Idle JR, Harris AL (1992) A phase I study on the reversal of multidrug resistance (MDR) in vivo: nifedipine plus etoposide. Br J Cancer 65: 267–270

27. Riva E, Gerna M, Latini R, Giani P, Volpi A, Maggioni A (1982) Pharmacokinetics of amiodarone in man. J Cardiovasc Pharmacol 4: 264–269
28. Riva E, Gerna M, Neyroz P, Urso R, Bartosek I, Guaitani A (1982) Pharmacokinetics of amiodarone in rats. J Cardiovasc Pharmacol 4: 270–275
29. Ro J, Sahin A, Ro JY, Fritsche H, Hortogagyl G, Blick M (1990) Immunohistochemical analysis of P-glycoprotein expression correlated with chemotherapy resistance in locally advanced breast cancer. Hum Pathol 21: 787–791
30. Samuels BL, Mick R, Vogelzang NJ, Williams SF, Schilsky RL, Safa AR, O'Brien SM, Ratain MJ (1993) Modulation of vinblastine resistance with cyclosporine: a phase I study. Clin Pharmacol Ther 54: 421–429
31. Sanfilippo O, Ronchi E, De Marco C, Di Fronzo G, Silvestrini R (1991) Expression of P-glycoprotein in breast cancer tissue and in vitro resistance to doxorubicin and vincristine. Eur J Cancer 27: 155–158
32. Schneider J, Bak M, Efferth T, Kaufmann M, Mattern J, Volm M (1989) P-glycoprotein expression in treated and untreated human breast cancer. Br J Cancer 60: 815–818
33. Sonneveld P, Durie BGM, Lokhorst HM, Marie JP, Solbu G, Suciu S, Zittoun R, Lowenberg B, Nooter K (1992) Modulation of multidrug-resistant multiple myeloma by cyclosporin. Lancet 340: 255–259
34. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S (1988) Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. Cancer Res 48: 1926–1929
35. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84: 7735–7738
36. Trump DL, Smith DC, Ellis PG, Rogers MP, Schold SC, Winer EP, Panella TJ, Jordan VC, Fine RL (1992) High-dose oral tamoxifen, a potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. J Natl Cancer Inst 84: 1811–1816
37. Van der Graaf WTA, deVries EGE, Uges DRA, Nanninga AG, Meijer C, Vellenga E, Milder POM, Mulder NH (1991) In vitro and in vivo modulation of multi-drug resistance with amiodarone. Int J Cancer 48: 616–622
38. Vrobel TR, Miller PE, Mostow ND, Rakita L (1989) A general overview of amiodarone toxicity: its prevention, detection, and management. Prog Cardiovasc Dis 31: 393–426
39. Yahanda AM, Adler KM, Fisher GA, Brophy NA, Halsey J, Hardy RI, Gosland MP, Lum BL, Sikic BI (1992) A phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 10: 1624–1634