Response of Human Breast Cancer Cells to Heat Shock and Chemotherapeutic Drugs

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ABSTRACT

Previous studies have shown that certain chemotherapeutic drugs are less effective on tumor cells when cells have been previously exposed to hyperthermia. In the present study, we have evaluated whether specific modifications in heat shock protein (hsp) expression are associated with resistance to anticancer drugs. RNA levels for hsp90, hsp70, and hsp27 were studied by Northern and slot blots, while proteins were studied by two-dimensional gel electrophoresis, in MCF-7/BK and MDA-MB-231 breast cancer cells. The sensitivities of these cells to doxorubicin, colchicine, 5-fluorouracil, cisplatin, actinomycin D, and methotrexate were tested by clonogenic assays. These techniques were applied to both cell lines (before and after heat shock). The study revealed that elevated hsp70 and hsp27 levels were associated with doxorubicin resistance. In addition, the presence of phosphorylated hsp27 isoforms was also associated with doxorubicin resistance. The study showed that elevated hsp27 were not associated with multidrug resistance. Heat shock did not induce P170 glycoprotein mRNA overexpression or resistance to the other drugs tested. We also found that the level of doxorubicin protection conferred by the overexpression of hsp was lower than that obtained in cells expressing a multidrug resistance phenotype (MDA-A, cells). In these cells, heat shock did not confer additional doxorubicin resistance and hsp27 phosphorylation was deficient. Our studies suggest that specific hsps are associated with doxorubicin resistance in certain human breast cancer cells and that this mechanism seems to be independent of the multidrug resistance system.

INTRODUCTION

Previous studies demonstrated more effective tumor cell cytotoxicity when hyperthermia was combined with simultaneous chemotherapy (1–3). However, when tumor cells were exposed sequentially to nonlethal elevated temperatures (heat shock) and then to chemotherapy, the latter was less effective (4, 5). This phenomenon has been observed for certain chemotherapeutic drugs only (doxorubicin, amphotericin B, and actinomycin D) (3, 6).

At the molecular level, at least three protein systems have been reported to be increased in stressed cells and involved in drug resistance: the grp, the Pgp, and the hsp. The grp system is coinduced with doxorubicin resistance in Chinese hamster cells (7). However, in mammalian cells the grp does not confer multidrug resistance (13) and drug resistance (14, 15).

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We regret to report that Dr. William L. McGuire died on March 25, 1992, after this manuscript was completed.

The abbreviations used are: grp, glucose-regulated protein; Pgp or P170, P-glycoprotein; mdr, multidrug resistance; hsp, heat shock protein; P-GAD-28, glyceraldehyde-3-phosphate dehydrogenase; IC50, drug concentration inhibiting colonies by 50%.

MATERIALS AND METHODS

Cell Culture and Maintenance. The breast cancer cell MDA-MB-231 was obtained from The American Type Culture Collection (Rockville, MD), and MCF-7/BK human breast cancer cells were kindly provided by Dr. B. Katzenellenbogen (University of Illinois). We also used a doxorubicin-resistant cell line, MDA-A, generated in our laboratory from the parent cell line MDA-MB-231 (16). All cell lines were maintained in Eagle’s medium supplemented with 10% fetal bovine serum, 6 ng/ml insulin, and 25 μg/ml gentamicin sulfate (Schering Corporation, Kenilworth, NJ), were cultured in T-150 flasks as continuous monolayer cell lines at 37°C in 5% CO2:95% air, and were periodically tested for Mycoplasma contamination (Bionique Laboratories, Saranac Lake, NY). In preliminary studies, we observed that MCF-7/BK cells had different responses to heat shock, according to their length in culture; for consistency, we utilized MCF-7/BK cells from passages 85 thru 115 (termed high passage). MDA-MB-231 and MDA-A, cells were from passages 40 thru 60. Clonogenic Assays. The different cell lines were grown in T-25 flasks and were used when approximately 70% confluent. Since some of the hsp are induced by estrogens (17, 18), 24 h before cell stimulation the standard culture medium was replaced with a phenol red-free medium supplemented with 10% charcoal-stripped fetal bovine serum (17). This minimizes exogenous estrogen or estrogen-like effects on heat shock proteins and on colony growth. We tested different soft agar concen-

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trations and growing media for MDA-MB-231 cells, because they did not grow as well as MCF-7/BK cells in soft agar. Optimum results were obtained with improved minimal essential medium and increased fetal bovine serum concentrations. Cloning efficiency for MDA-MB-231 cells was 18% and for MCF-7/BK cells was 38%. Twenty-four h before experiments, MDA-MB-231 cells were placed in improved minimal essential medium (phenol red-free) supplemented with 15% charcoal-stripped fetal bovine serum. In the initial experiments, we also observed severe cell killing in MCF-7/BK cells after 43°C or higher heat shock. Therefore, we also tested different heat shock conditions, and 2 h at 42°C was selected as our optimum heat shock treatment.

For the clonogenic assays, control cells growing at 37°C and heat-shocked cells (42°C for 2 h, followed by a resting period of 4 h at 37°C) were exposed at 37°C for 1 h to varying concentrations of doxorubicin hydrochloride (Adria Laboratories Inc., Columbus, OH), colchicine (Sigma), 5-fluorouracil (Hoffmann-LaRoche Inc., Nutley, NJ), cisplatin (Bristol Laboratory, Evansville, IN), actinomycin D (Cosmegen; Merck, Sharp, and Dohme, West Point, PA), or methotrexate (Lederle Parenterals, Inc., Carolina, Puerto Rico). The cells were then pelleted, washed in phosphate-buffered saline, resuspended in stripped growing medium, and plated in soft agar as described (19), with minor modifications. Briefly, a bottom layer of 0.6% agarose (FMC BioProducts, Rockland, ME) for MDA-MB-231 cells or 0.5% agarose for MCF-7/BK cells was prepared in 35-mm Petri dishes (Sarstedt, NJ). A 1 ml top layer containing 2.5 × 10^6 cells in 0.4% agarose (for MDA-MB-231 cells) or 0.6% agarose (for MCF-7 cells) was layered on top of the hardened bottom layer. All experiments were done in duplicate. The dishes were incubated in high humidity incubators (37°C) for 7 to 10 days, and colonies measuring ≥100 µm were counted with an inverted microscope. Percentage survival was defined as the number of colonies in drug-treated cultures, compared to the number of colonies in untreated controls. Drug resistance was measured by determining the IC₅₀ for each tested condition. Percentage of cells in S-phase fraction was studied by flow cytometry, as described elsewhere (20). This study was carried out on MCF-7/BK and MDA-MB-231 cells, before heat shock (control) and after heat shock (42°C for 2 h, followed by a resting period of 4 h at 37°C).

RNA Analyses. Total cellular RNA was extracted from control and heat-shocked cells, following the manufacturer's recommendations, on a model 340A nucleic acid extractor (Applied Biosystems, Inc., Foster City, CA). For Northern blots, 10 µg of RNA, quantitated spectrophotometrically, were electrophoresed on a 1% agarose/17% formaldehyde gel and transferred to Nytran membranes (Schleicher and Schuell, Inc., Keene, NH). For slot blots, 10 µg of RNA were applied to Nytran membranes using a MiniFold II slot blotter (Schleicher and Schuell). The following probes were used for hybridization: hsp27 (21), hsp70 (22), hsp90α (kindly provided by Dr. Lee A. Weber, Biology Department, University of South Florida, Tampa, FL) (23), hsp90β (kindly provided by Dr. Neil F. Rebbe, Washington University Medical Service, Veterans Affairs Medical Center, John Cochran Division, St. Louis, MO) (24), grp78 (25), P170 (16), and P4GAD-28 (26). The latter was used to demonstrate equivalent RNA loading and transfer. Hybridization was performed as described previously (21). Briefly, probes were labeled with 32P by random-primed DNA labeling (27), and probes and membranes were placed in sealed plastic bags at 42°C (grp78 and P4GAD-28) or at 52°C (hsp probes and P170) for 16 h. Hybridization solutions were 50% formamide, 1× Denhardt's solution [prepared from a 50× Denhardt's reagent: 5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g bovine serum albumin (Fraction V), and H₂O to 500 ml], 0.5% sodium dodecyl sulfate, 5× standard saline/sodium phosphate/EDTA (prepared from a 20× SSPE: 87.65 g NaCl, 13.8 g Na₂HPO₄, 3.7 g EDTA, H₂O to 500 ml, pH 7.4 (with 10 mM NaOH)). 100 µg/ml denatured salmon sperm DNA (grp78 and P4GAD-28), or 50% formamide, 5× standard saline/sodium phosphate/EDTA, 1× Denhardt's solution, 0.5% sodium dodecyl sulfate, 100 µg/ml salmon sperm DNA (hsp probes and P170). After hybridization, the membranes were washed (21) and autoradiographed. Relative RNA induction was determined by scanning densitometry of RNA signals, normalized against signals obtained with the P4GAD-28 probe. Ribonuclease protection assays were performed with a human mdr-1 complementary DNA insert in pGEM-4 (Promega, Madison, WI) (16), linearized with PvuII. An RNA probe was synthesized with a Riboprobe kit (Promega), according to the manufacturer's instructions, using the T7 promoter. This resulted in a probe size of approximately 400 base pairs, containing 324 base pairs of the mdr-1 coding sequence and 76 base pairs of vector sequences. The probe 36B4 (29), digested with RsaI, was used as a control for loading. The 36B4 complementary DNA protects a fragment of 145 base pairs from RNase digestion. Thirty µg of total RNA were hybridized as previously described (30) and electrophoresed on a 5% polyacrylamide gel containing 8 M urea. The gel was dried, exposed to X-ray film for 1 to 3 days, and quantitated by densitometry.

Protein Analyses. Cells were mechanically harvested and washed in phosphate-buffered saline. An aliquot was taken for protein determination by the bichinonic acid method (Pierce, Rockford, IL) (31), and the remaining cells were pelleted and prepared for high-resolution two-dimensional gel electrophoresis, as described elsewhere (32). After electrophoresis, proteins were transferred from the gels to nitrocellulose membranes using electrot blotting techniques (33). Following blocking of nonspecific sites with 5% nonfat dry milk (1 h), the blots were incubated with a mixture of mouse monoclonal antibodies against hsp27 (21), hsp70 (N27F3-4) (34), and hsp90 (AC88) (35). The blots were incubated overnight at 4°C with the primary antibodies (2 µg/ml) and, after washing, 125I-labeled sheep anti-mouse IgG (100,000 cpm/ml; Amersham, Arlington Heights, IL) was used as the secondary antibody. The blots were washed and exposed for 1–3 days to X-OMAT X-ray film (Kodak, Rochester, NY), at −70°C, using intensifying screens.

hsp phosphorylation was studied by incubating the cells in serum- and phosphate-free tissue culture medium for 45 min and changing medium to fresh medium containing 100 µCi/ml [32P]H₃PO₄ (carrier free) in water (New England Nuclear, Boston, MA). After 40 min, the cells were divided into two groups: (a) control (maintained at 37°C) and (b) heat shock treated (42°C for 20 min or 2 h). After this, the cells were washed in cold phosphate-buffered solution (8 g NaCl, 0.2 g KCl, 1.15 g Na₂HPO₄, 0.2 g KH₂PO₄, and H₂O to 1 liter), and the proteins were extracted for two-dimensional gel electrophoresis. Proteins were then transferred to nitrocellulose membranes for autoradiography. To demonstrate the exact localization of the hsp, the membranes were then incubated with a mixture of antibodies against hsp27, -70, and -90. The antigen-antibody complexes were visualized by immunostaining with biotinylated secondary antibody, followed by streptavidin/biotinylated horseradish peroxidase reagents (Dako Corporation, Santa Barbara, CA) and diaminobenzidine tetrahydrochloride (Sigma) (36).
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Fig. 1. Composite figure showing Northern blot analyses of MDA-MB-231 cells. No changes were observed in the expression of pGAD-28 (1.6 kilobases) in control (C) and heat-shocked (HS) cells. hsp27 mRNA (0.9 kilobase) was significantly induced in heat-shocked cells, while absence of induction was also obtained for grp78 (2.6 kilobases) mRNA after heat shock. mRNA sizes were determined using a Bethesda Research Laboratory (Rockville, MD) RNA size ladder.

Fig. 2. RNase protection assay using control and heat-shocked breast cancer cells. Two probes were used, P170p and 36B4p, and they are shown in Lane 1. The samples with the protected fragments P170f and 36B4f are in Lanes 2 through 7. Lane 2, MDA-A, control; Lane 3, MDA-A, heat shock; Lane 4, MDA-MB-231, control; Lane 5, MDA-MB-231, heat shock; Lane 6, MCF-7/BK, control; Lane 7, MCF-7/BK, heat shock. Sizes of the standards used are shown on the left (404, 309, 217, 180, 160, and 147 base pairs).

revealed that hsp27 mRNA was overexpressed in MCF-7/BK cells grown at 37°C, compared to MDA-MB-231 cells (Fig. 3).

Different hsp Isoform Patterns in MDA-MB-231 and MCF-7/BK Cells. In order to determine the hsp protein isoform levels in the two cell lines as a function of heat induction, protein extracts from control and heat-shocked cells were subjected to two-dimensional gel electrophoresis (Fig. 4). Different hsp isoform patterns were observed not only after heat shock but also when control MDA-MB-231 cells were compared with control MCF-7/BK cells. MDA-MB-231 cells growing at control temperature showed discrete basal hsp27, -70, and -90 patterns. After heat shock, a notable increase in hsp27 and hsp70 expression levels was noted (Fig. 4). In contrast, MCF-7/BK cells at control temperature showed higher basal hsp70 and hsp27 levels than did MDA-MB-231 cells growing at the same temperature. In addition, the acidic hsp27B isoform was observed in MCF-7/BK cells grown at 37°C; MDA-MB-231 cells induced this isoform only with heat shock. The isoform pattern for hsp27 remained unchanged in heat-treated MCF-7/BK cells.

Phosphorylation patterns for the three hsp27 studied are shown in Fig. 5. A comparison of the spots obtained after 32P incorporation (Fig. 5, left) and the spots obtained after specific monoclonal antibody incubation (Fig. 5, right) revealed that hsp90 and hsp27 were phosphorylated, but not hsp70. In addition, this study revealed that the acidic hsp27 isoforms (B and C) were phosphorylated; hsp27 isoform A was not phosphorylated even after heat shock treatment. hsp27 phosphorylation represented almost 30% of total hsp27 (after spot quantitation). The phosphorylation pattern for MDA-MB-231 cells was similar to that obtained for MCF-7/BK cells, except for the absence of hsp27 phosphorylation in the control cells.

Heat Shock Induction of Doxorubicin Resistance but Not Multidrug Resistance. Heat shock increased doxorubicin resistance in MDA-MB-231 and MCF-7/BK cells, but only when a recovery period at 37°C was allowed before drug exposure (Fig. 6). MCF-7/BK cells were significantly more sensitive to doxorubicin when exposed to the drug immediately after heat shock. MDA-MB-231 cells were 11-16-fold more resistant to doxorubicin after heat shock, while MCF-7/BK cells displayed a 2.5-3-fold doxorubicin resistance following heat shock (Table 1). However, at 37°C MCF-7/BK cells (cells with higher hsp70 and hsp27) were approximately 7-fold more resistant to doxorubicin than were MDA-MB-231 cells. Therefore, only after heat shock, these cell lines showed both equivalent absolute levels of resistance to doxorubicin (IC50 ~ 0.3-0.4 µg/ml) and similar hsp patterns. We also tested other drugs by soft agar clonogenic assays. MCF-7/BK and MDA-MB-231 cells did not show significant resistance to colchicine, cisplatin, 5-fluorouracil, actinomycin D, or methotrexate following heat shock (Table 1).

A cell kinetic study was performed to determine whether heat shock had taken the cells out of the S-phase of the cell cycle, thus affecting drug resistance. Our heat shock treatment did...
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Fig. 3. Composite figure showing slot blot analyses of MDA-MB-231 and MCF-7/BK cells before (control) and after heat shock. The probe to detect pGAD-28 was used to compare sample loading. Note the induction of hsp mRNAs after heat shock. Overexpression of hsp27 mRNA is also observed in control MCF-7/BK cells (compare with hsp27 mRNA expression in control MDA-MB-231 cells).

Fig. 4. Two-dimensional gel electrophoresis, followed by Western blot, of MDA-MB-231 and MCF-7/BK cells (loading equal amounts of protein sample). Heat shock proteins were detected using a cocktail of monoclonal antibodies. Note the expression of more hsp70 (hsp73/72) and hsp27 (including the acidic isoform B) in control MCF-7/BK than in MDA-MB-231 cells. A discrete hsp90 protein signal was obtained; this is not the total hsp90 mass protein and may be due to the antibody or the special methods used here. After heat shock, acidic hsp27 isoforms (C and B) were observed in MDA-MB-231 cells, together with more hsp90 and hsp70. In contrast, MCF-7/BK cells showed a moderate increase in hsp27 after heat shock.

Fig. 5. Protein phosphorylation pattern of MCF-7/BK cells growing at control temperature (upper) and after heat shock (lower). The proteins were separated by two-dimensional gel electrophoresis and transferred to nitrocellulose paper. Phosphoproteins (left) were revealed by exposure to X-ray film, while heat shock proteins (right) were detected using a cocktail of monoclonal antibodies. Circles (left), place for hsp70 (nonphosphorylated proteins). hsp90 and hsp27 (acidic isoforms B and C) were phosphorylated.

MDA-MB-231 cells. We began the study by characterizing hsp levels and the response to doxorubicin in our MCF-7/BK and MDA-MB-231 cells when they were growing at control temperature, showing that untreated (37°C) MCF-7/BK cells displayed not only an increase in hsp27 mRNA and protein but also hsp27 phosphorylation. We are studying the factors that may be involved in the constitutive overexpression of hsp27 mRNA and protein in our MCF-7/BK cells. Previous studies have shown, in nonmammary cells, hsp27 phosphorylation after heat shock and other stresses (37-40). In MCF-7 and MDA-231 cells, Regazzi et al. (41 ) have shown hsp27 phosphorylation induced by phorbol-12-myristate-13-acetate administration; however, their MCF-7 cells did not display hsp27 phosphorylation under control conditions. The degree of hsp27 phosphorylation seems to determine the ability of the cells to survive hyperthermia (40). Although our study was not designed to investigate thermotolerance, when we were defining our heat shock conditions (see "Materials and Methods") we observed severe cell killing for MCF-7/BK cells exposed to temperatures above 43°C. High hsp27 protein levels have been associated

not decrease significantly the percentage of cells in S-phase (Table 2).

The effect of heat shock on doxorubicin resistance was also tested in our multidrug-resistant MDA-A111 cell line (16) (Fig. 6). Doxorubicin resistance was not increased to a greater extent after heat shock in MDA-A111 cells. Although not shown in Fig. 6, we tested even higher doxorubicin concentrations. The IC50 values were not different between control and heat-shocked MDA-A111 cells. Since these cells showed a high level of doxorubicin resistance at 37°C, it was of interest to know the hsp isoform patterns of these cells (Fig. 7). In this case, the film was exposed longer, to detect hsp27 acidic isoforms; however, it was clear that the phosphorylated form of hsp27 was not induced in these cells. On the other hand, they showed induction of hsp90 and hsp70 after heat shock.

DISCUSSION

This study shows that elevated hsp70 and hsp27 levels correlate with doxorubicin resistance in MCF-7/BK cells and
while hsp27 interacts with microfilament proteins, inhibiting actin polymerization (46,47). Therefore, at present it is difficult to correlate these roles with doxorubicin resistance, especially because this drug exhibits several mechanisms by which it may damage cells (48).

On the other hand, our study also shows that elevated hsp70 and hsp27 levels are not associated with typical multidrug resistance. Heat shock increased doxorubicin resistance in the two cell lines studied; however, they did not display increased resistance to the other drugs tested. Previous investigators have found that cells treated with heat shock or transfected with a human hsp27 gene showed resistance not only to doxorubicin but also to drugs such as colchicine, actinomycin D, and vincristine (4, 6, 15). In the present study, we were unable to observe chemoresistance to these drugs following heat shock. At present, we may only speculate that this difference in drug resistance may be due to differences in the cells studied and in the experimental conditions used.

Doxorubicin is generally more active in cells in S-phase; our cell kinetic study revealed that heat shock was not significantly affecting this fraction. Moreover, resistance to methotrexate, another S-phase-specific drug, was not affected by heat shock. These results indicate that doxorubicin resistance following heat shock was not due to modification of the cell cycle.

It is important to mention here that our MCF-7/BK and MDA-MB-231 cells did not show induction of the grp or mdr-1 systems following heat shock. However, we cannot rule out the possibility that heat exposure could alter other protein systems which may also contribute to drug resistance (e.g., glutathione reduct or glutathione peroxidase). Future studies will be needed to clarify this issue. In addition, we found that the level of doxorubicin protection conferred by the overexpression of hsp is much lower than that obtained with Pgp overexpression. This was clearly seen when MDA-A16 cells were studied at 37°C and after heat shock. These cells, selected for doxorubicin resistance, express a multidrug-resistant phenotype (16). Heat shock was unable to confer additional resistance to these cells, perhaps due to the fact that they showed very little hsp27 phosphorylation after heat shock. A previous study has shown that the response was evaluated by comparing the IC50 after heat shock with the IC50 of the control untreated cells.

The mechanisms for the acquisition of doxorubicin resistance by hsp70 and hsp27 are unclear. It seems that this is not due to a decrease in drug accumulation, since cells transfected with the hsp27 gene that became resistant to daunorubicin did not show lower drug accumulation than the parental cells (15). hsp70 is involved in protein translocation across different cellular membrane compartments, stabilizing unfolded proteins (42–45), while hsp27 interacts with microfilament proteins, inhibiting actin polymerization (46,47). Therefore, at present it is difficult to correlate these roles with doxorubicin resistance, especially because this drug exhibits several mechanisms by which it may damage cells (48).

We then explored the hsp levels and the response to doxorubicin after heat shock in these cells. Increased doxorubicin resistance was found in both cell lines, but more drug resistance was induced in the cell line shown in Fig. 1. Total hsp27 levels were higher in both cell lines, but expression levels after heat shock (MDA-MB-231). Little in-crease in hsp27 (mRNA, total mass of protein, and phosphorylation) was observed after heat shock in MCF-7/BK cells, and resistance to the other drugs tested. Previous investigators have found that cells treated with heat shock or transfected with a human hsp27 gene showed resistance not only to doxorubicin but also to drugs such as colchicine, actinomycin D, and vincristine (4, 6, 15). In the present study, we were unable to observe chemoresistance to these drugs following heat shock. At present, we may only speculate that this difference in drug resistance may be due to differences in the cells studied and in the experimental conditions used.

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**Table 1** Sensitivity of breast cancer cells to chemotherapeutic drugs after heat shock treatment

<table>
<thead>
<tr>
<th>Drug tested</th>
<th>Cell line</th>
<th>IC50 (μg/ml)</th>
<th>Heat shock/control</th>
<th>Response*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>MCF-7/BK</td>
<td>0.43/0.17</td>
<td>2.5X resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>0.35/0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colchicine</td>
<td>MCF-7/BK</td>
<td>0.4/0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>0.085/0.089</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>MCF-7/BK</td>
<td>55/77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>70/68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>MCF-7/BK</td>
<td>85/76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>100/89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>MCF-7/BK</td>
<td>0.083/0.067</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>0.80/0.86</td>
<td></td>
<td></td>
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<tr>
<td>Methotrexate</td>
<td>MCF-7/BK</td>
<td>1.7/2.2</td>
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<td></td>
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</tbody>
</table>

* The response was evaluated by comparing the IC50 after heat shock with the IC50 of the control untreated cells.

**Table 2** Kinetics of MCF-7/BK and MDA-MB-231 cells analyzed by flow cytometry

<table>
<thead>
<tr>
<th>Cell line</th>
<th>G0-G1</th>
<th>S</th>
<th>G2-M</th>
</tr>
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<tbody>
<tr>
<td>MCF-7/BK, control</td>
<td>46.15</td>
<td>40.36</td>
<td>13.49</td>
</tr>
<tr>
<td>MCF-7/BK, heat shock</td>
<td>45.69</td>
<td>36.31</td>
<td>18.00</td>
</tr>
<tr>
<td>MDA-MB-231, control</td>
<td>51.73</td>
<td>36.92</td>
<td>11.35</td>
</tr>
<tr>
<td>MDA-MB-231, heat shock</td>
<td>48.45</td>
<td>37.50</td>
<td>14.05</td>
</tr>
</tbody>
</table>
pointed out that cells selected for doxorubicin resistance were not cross-resistant to heat and that they did not exhibit drug potentiation by hyperthermia (5). Therefore, it seems that in MDA-AiR cells doxorubicin resistance is completely independent of the heat shock response. Even when the levels of doxorubicin resistance after heat shock were lower than those observed in cells expressing the multidrug resistance phenotype, the involvement of hsps in drug resistance may be clinically relevant. The resistance phenomenon associated with hsps may be permanent (overexpression due to genetic alterations) or transient, it seems to be selective for certain drugs, and it may be triggered not only by heat shock but also by other stresses that usually occur in human tumors.

At present, very few studies have been done on hsps in human breast cancer. Only hsp27 has been evaluated (49, 50) and, since this is an estrogen-regulated protein (17, 21), the efforts have been directed toward determining its association with estrogen receptors (51–53). Lately, the expression of this hsp has also been evaluated as a prognostic factor (54–56). In light of the association of hsps with drug resistance, in the future it will be of interest to explore whether overexpression of hsps is associated with clinical drug resistance in breast cancer patients.

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