

Accelerated Publications

Defective pH Regulation of Acidic Compartments in Human Breast Cancer Cells (MCF-7) Is Normalized in Adriamycin-Resistant Cells (MCF-7adr)[†]

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ABSTRACT: Alkalinization of normally acidic intracellular compartments or acidification of a mildly alkaline cytoplasm by biochemical or genetic manipulation has been demonstrated to inhibit both endocytosis and secretion (Tartakoff, 1983a; Cosson et al., 1989; Mellman et al., 1986; Davoust et al., 1987; Cosson et al., 1989; van Deurs et al., 1989; Maxfield & Yamashiro, 1991; Hansen et al., 1993). These results provide the basis for the conclusion that the maintenance of pH gradients between acidic vesicular compartments and a mildly alkaline cytoplasm is an essential biochemical requirement for the correct functioning of the endocytotic and secretory machinery. Tumor cells have been shown to have an abnormally acidic cytoplasmic pH (Warburg, 1956; Simon & Schindler, 1994). Here we report that the intracellular vesicular compartments in tumor cells (MCF-7) derived from a human breast cancer fail to acidify. This failure results in a significant decrease in the pH gradient (0.9 pH unit) between the vesicular luminal compartments and the cytoplasm. These defects are correlated with a disruption in the organization and function of the *trans*-Golgi network (TGN) and the pericentriolar recycling compartment (PRC). In marked distinction, drug-resistant tumor cells (MCF-7adr) derived from the MCF-7 line that are resistant to the most widely employed chemotherapeutic drug, adriamycin, appear normal in both acidification and organization of the PRC and TGN. Treatment of drug-resistant MCF-7adr cells with nigericin and monensin, ionophores demonstrated to disrupt vesicular acidification (Tartakoff, 1983b), leads to a resensitization of these cells to adriamycin. Drug sensitivity is proposed to result from an acidification defect within vesicles of the recycling and secretory pathways. A functional consequence of this defect is the diminished capacity of cells to remove cytotoxic drugs from the cytoplasm by sequestration of protonated drugs within the vesicles, followed by drug secretion through the activity of the secretory and recycling pathways.

Cancer cells are more sensitive to chemotherapeutic drugs than normal cells. However, tumor cells treated with

chemotherapeutics often develop a resistance to these agents that is accompanied by changes in tumor cell physiology. Such resistant cells display properties that include overexpression of numerous cellular proteins, changes in the subcellular distribution of the chemotherapeutics, and an alkaline shift of cytoplasmic pH (Simon & Schindler, 1994).

Since most of the chemotherapeutics are weak bases with pKs of 7–8, it has been proposed that the alkaline shift could be causally related to drug resistance (Warburg, 1956; Dalmark & Hoffmann, 1983; Beck, 1987; Sehested et al., 1988; Roepe et al., 1994; Simon & Schindler, 1994). Thus,

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Table 1: Calibrated Average pH^a

compartment	MCF-7	MCF-7adr (MDR)	epithelial (CFTR mutant) (Barasch et al., 1991)	epithelial (wt) (Barasch et al., 1991)	endosomes (1 mM Cl ⁻)	endosomes (140 mM Cl ⁻) (Van Dyke & Belcher, 1994)
organelle	6.5 ^b	5.9 ^b	6.8	6.3	6.3 ^c	5.8 ^c
cytosol	6.8	7.1	7.0	7.0	7.0 ^d	7.0 ^d
ΔpH (organelle/cytosol)	0.3	1.2	0.2	0.7	0.7	1.2
Δ(ΔpH) gradient		0.9		0.5		0.5

^a The first two columns of pH measurements for the MCF-7 and MCF-7adr cells are from this work. The third and fourth columns are measurements from two cells expressing either the mutant (column 3) or wt (column 4) CFTR (Barasch et al., 1991). The ionic basis for the acidification in the organelles was examined by resuspending them in media of varying ionic concentration (Van Dyke & Belcher, 1994). The effects of varying the chloride concentration are shown in column 5 (absence of chloride) and column 6 (in the presence of 140 mM Cl⁻) (Van Dyke & Belcher, 1994).

^b Weighted mean calculated from Figure 2. ^c Average pH determined for multivesicular vesicles, CURL, and recycling compartments (Van Dyke & Belcher, 1994). ^d pH of buffer.

chemotherapeutics would be expected to accumulate in the more acidic cytoplasm of tumor cells (Simon et al., 1994). The pH difference between many drug-resistant tumor cells and their parental cell lines is sufficient to quantitatively account for the decreases in drug accumulation observed in drug-resistant cells (Simon et al., 1994). However, it has recently been reported for one particular cell line, the MCF-7 breast cancer cell line, that the difference in cytoplasmic pH between the drug-resistant and drug-sensitive cells is only 0.2 pH unit (Altenberg et al., 1993). This modest pH differential is considered too small to account for the differences in cellular drug accumulation.

Here we describe structural and functional alterations within breast cancer cell lines that are correlated with either enhanced drug sensitivity (MCF-7) or resistance (MCF-7adr). These differences are observed as (a) changes in the organization and activity of the secretory compartment (TGN), (b) disruption of the structure and activity of the pericentriolar recycling compartments (PRC), and (c) alterations in both cytoplasmic and vesicular pH. The data support the view that drug resistance occurs through the reacquisition of pH gradients between the cytoplasm and the luminal compartments of the TGN and endosomes. Such pH gradients are necessary for the proper functioning of the recycling and secretory pathways (Tartakoff, 1983a; Mellman et al., 1986; Davoust et al., 1987; Cosson et al., 1989; van Deurs et al., 1989; Maxfield & Yamashiro, 1991; Hansen et al., 1993).

EXPERIMENTAL PROCEDURES

Reagents. Acridine orange was purchased from Aldrich (Milwaukee, WI). The fluorescent reagents, Bodipy-ceramide, the acetoxymethyl esters of both carboxy SNARF-1 and SNAFL-calcein, and Bodipy-lactalbumin, were from Molecular Probes (Eugene, OR). Adriamycin was from Calbiochem (La Jolla, CA). Monensin and nigericin were from Sigma (St. Louis, MO).

Tissue Culture. Cells were seeded and grown in Dulbecco's Modified Eagle's (DME) medium containing 10% fetal calf serum (phenol red free) in Lab-Tek culture chambers (Nunc, Naperville, IL) maintained in an incubator at 37 °C and 5% CO₂. Human breast cancer cells (MCF-7) and the adriamycin-resistant line (MCF-7adr) were obtained from Dr. William W. Wells of the Department of Biochemistry, Michigan State University. The media for the MCF-7adr

cells were supplemented with adriamycin (0.5 μg/mL). Cells were utilized for 3–4 days following plating.

Confocal Fluorescence Imaging of Intracellular pH and Acidic Compartments. Acridine orange (2 μg/mL media; 4 mg/mL stock in water) was added directly to the chambers, and the cells were incubated with the dye at 37 °C for 30 min. Cells in the presence of acridine orange were then examined at room temperature with an Insight bilateral laser scanning confocal microscope (Meridian Instruments, Okemos, MI). Excitation was at 488 nm (argon ion laser beam), and dual emission confocal images were sequentially recorded utilizing both a 530–30 band-pass barrier filter (green fluorescence) and a 605 nm long-pass barrier filter (red fluorescence). Acridine orange demonstrates a concentration-dependent long-wavelength shift in the fluorescence emission; it shows a red fluorescence when accumulated to a high concentration within acidic cellular compartments and a green fluorescence when bound at a lower concentration to membranes and/or nucleic acids. Optical sections of the fluorescent sample were recorded at 0.5 μm intervals. Typical individual sections are presented to demonstrate the distribution of acridine orange within the cytoplasmic and vesicular compartments. The acetoxymethyl ester derivative of either carboxy SNARF-1 or SNAFL-calcein (15 μg/mL) (Molecular Probes, Eugene, OR) (ratiometric fluorescent probes for pH) was added to both MCF-7 and MCF-7adr cells. The ester-linked fluorescent probes enter the cell passively where the esters are hydrolyzed by esterases located in the cytoplasm and intracellular vesicles. The carboxy SNARF-1 or SNAFL-calcein is then ionically trapped within the cytoplasm (carboxy SNARF-1) or vesicular (SNAFL-calcein) compartments. The cells were incubated at 37 °C for 45 min and then examined with the Insight confocal microscope. Optical sections were obtained utilizing two different filter settings for emission (530–30 band-pass barrier filter and 630 long-pass filter) and a single excitation wavelength (488 nm) as previously described for carboxy SNARF-1 (Simon et al., 1994). To obtain cytosolic pH, carboxy SNARF-1 emission ratios were determined at different sites throughout the cytoplasm. These areas demonstrated an isotropic distribution of dye. The pixel intensities obtained at the two different emission wavelengths were divided to obtain a ratio image of the internalized pH probe (Simon et al., 1994). These images were then compared to standard curves that were obtained in the following manner. To obtain a quantitative relationship

between emission ratios and pH, each SNAFL-calcein stained cell line was exposed to a buffer at a known pH containing nigericin/high K^+ ($18 \mu\text{M}/150 \text{ mM KCl}$) (Simon et al., 1994). This treatment equilibrates all the internal compartments of the cell to the pH of the incubating buffer. By sequentially changing the pH buffer that is bathing the cells, a pH curve was generated for each cell line that demonstrated the relationship between the SNAFL-calcein fluorescence emission ratio and pH. These values were then incorporated into a pH imaging routine that provides a direct readout of pH values for individual intracellular compartments that are queried on the computer screen. Cells treated with monensin ($2 \mu\text{M}$) were exposed for 30 min at 37°C prior to labeling with SNAFL-calcein as described above. All cells were examined at room temperature. This concentration of monensin, by itself, does not significantly affect the viability of the cells but can sensitize cells to adriamycin (Figure 4).

Fluorescence Labeling and Confocal Imaging of Endosomal and Secretory Compartments. Bodipy-ceramide (Bodipy-Ceramide; Molecular Probes, Eugene, OR) has been demonstrated to label Golgi compartments (Pagano et al., 1989). Conversion of Bodipy-ceramide to Bodipy-sphingomyelin (in *cis*-Golgi) is associated with the movement of the newly synthesized fluorescent lipid to the *trans*-Golgi network (TGN) (Pagano et al., 1989). As the concentration of Bodipy-sphingomyelin increases within the TGN and secretory vesicles, a long-wavelength shift in fluorescence occurs that results in red fluorescent structures (TGN and secretory vesicles) against a green fluorescent background. Cells were incubated with Bodipy-ceramide ($3 \mu\text{g}/\text{mL}$) for 15 min at 37°C , washed once with fresh media, and then examined in optical sections at room temperature with confocal fluorescence microscopy. Excitation was at 488 nm, and dual emission images were prepared utilizing the filter set described for acridine orange (Figure 1). To examine internalization, Bodipy-lactalbumin (Bodipy-Lac; Molecular Probes, Eugene, OR) was used as a fluid-phase marker. Cells were incubated with Bodipy-Lac ($2 \text{ mg}/\text{mL}$) for 90 min at 37°C and then washed once with cold media and rapidly examined with confocal fluorescence microscopy ($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 530\text{--}30 \text{ nm}$ band-pass filter).

Cell Viability Assays. The media were removed 60 h after plating the cells and replaced with fresh media supplemented with various concentrations of adriamycin (Calbiochem, CA) and monensin (dissolved in DMF, 0.1%) (Sigma, St. Louis). After 6 h the media were removed; the cells were rinsed and then fed with fresh media not containing drugs. The cells were fed daily for 3 days, and then the DNA content of the adherent cells was quantified fluorometrically by Hoechst 33258 fluorescence. Media were aspirated and the wells rinsed with Hanks balanced salt solution (HBSS, phenol red free). The cells were sonicated in hypotonic media ($0.1 \times \text{HBSS}$) for 30 s. The homogenate from each well was collected, and Hoechst 33258 was added to a final concentration of $1 \mu\text{g}/\text{mL}$. Fluorescence was measured on an SLM Aminco-Bowman series 2 luminescence spectrometer with a $\lambda_{\text{ex}} = 356 \text{ nm}$ and a $\lambda_{\text{em}} = 492$. Calf thymus DNA was used for calibration.

RESULTS AND DISCUSSION

The fluorescent pH-sensitive probe carboxy SNARF-1 (acetoxymethyl ester form) (Whitaker et al., 1991) (Molec-

ular Probes, Eugene, OR) was employed to measure the intracellular pH in both MCF-7 and MCF-7adr cells. This probe partitioned within the cytoplasm of both cell lines, demonstrating little internalization within intracellular compartments. The cytoplasmic pH for MCF-7 cells was 6.8 ± 0.1 (10 cells, three separate confocal sections) and for MCF-7adr cells 7.1 ± 0.1 (10 cells, three separate confocal sections) (Table 1), consistent with other published measurements reporting a more acidic cytoplasm for drug-sensitive cells (Simon & Schindler, 1994). The more acidic cytoplasmic pH measured in MCF-7 cells suggested that the drug-sensitive cells were manifesting an aberrant regulation of intracellular pH that might be representative of other changes in pH, particularly within intracellular vesicular compartments. This was examined with acridine orange, a probe previously employed to establish the presence of acidic intracellular compartments (Barasch et al., 1991). As observed in Figure 1, MCF-7 cells have few orange-stained vesicles within the cytoplasm (Figure 1, top row left). In sharp contrast, intensely-red stained vesicles are observed in MCF-7adr cells in the pericentriolar region of the cytoplasm and dispersed throughout the cytoplasm (Figure 1, top row right). Treatment of either cell type with nigericin ($7.5 \mu\text{M}$) eliminated fluorescent staining of both cytoplasmic vesicles and vesicles within the pericentriolar region (Figure 1, second row), the same asymmetric cellular localization observed for the acidic compartments of the TGN and the PRC (Presley et al., 1993; McGraw et al., 1993).

To quantify the pH in intravesicular compartments, we used the fluorescent pH probe SNAFL-calcein (acetoxymethyl ester form) (Whitaker et al., 1991). The protonated and neutral forms of SNAFL-calcein were observed to differentially localize within the two cell types. In MCF-7 cells, the probe was found evenly distributed in its protonated (green) and neutral (red) forms between the cytoplasm and vesicular compartments (Figure 1, bottom row left). Again, in sharp contrast, in the MCF-7adr cells, the SNAFL-calcein, found predominantly localized to vesicles within the pericentriolar region, was significantly more protonated (green) and the cytoplasm was somewhat more alkaline (red) (Figure 1, bottom row right). This is similar to the subcellular distribution of labeled compartments observed with acridine orange (Figure 1, top row). To quantify the pH within these vesicular compartments, the fluorescence emission ratio of the acidic and neutral probes of SNAFL-calcein was determined for individual vesicles within a population of MCF-7 and MCF-7adr cells (20 cells of each cell type) (Simon et al., 1994). These values were then compared to a standard curve (see legend to Figure 1). As shown in the pH histogram (Figure 2, Table 1), on average the vesicular compartments are more acidic in MCF-7adr cells than in the sensitive MCF-7 cells. This results in pH gradients between the cytoplasm and the luminal compartments that are considerably larger in MCF-7adr cells (Table 1). These measurements indicate that drug-sensitive MCF-7 cells do not contain significant numbers of acidified lysosomes (4.8–5.2), late endosomes (pH 5.2–5.8), sorting endosomes (pH ~6.0), endosomes (pH ~6.0–6.3) (Mellman et al., 1986; Maxfield & Yamashiro, 1991; van Deurs et al., 1989), or secretory vesicles (pH ~5.8) (Russell & Holz, 1981). In this regard, the data in Figure 2 and Table 1 argue that the MCF-7adr line more closely resembles normal, nontumor cells.

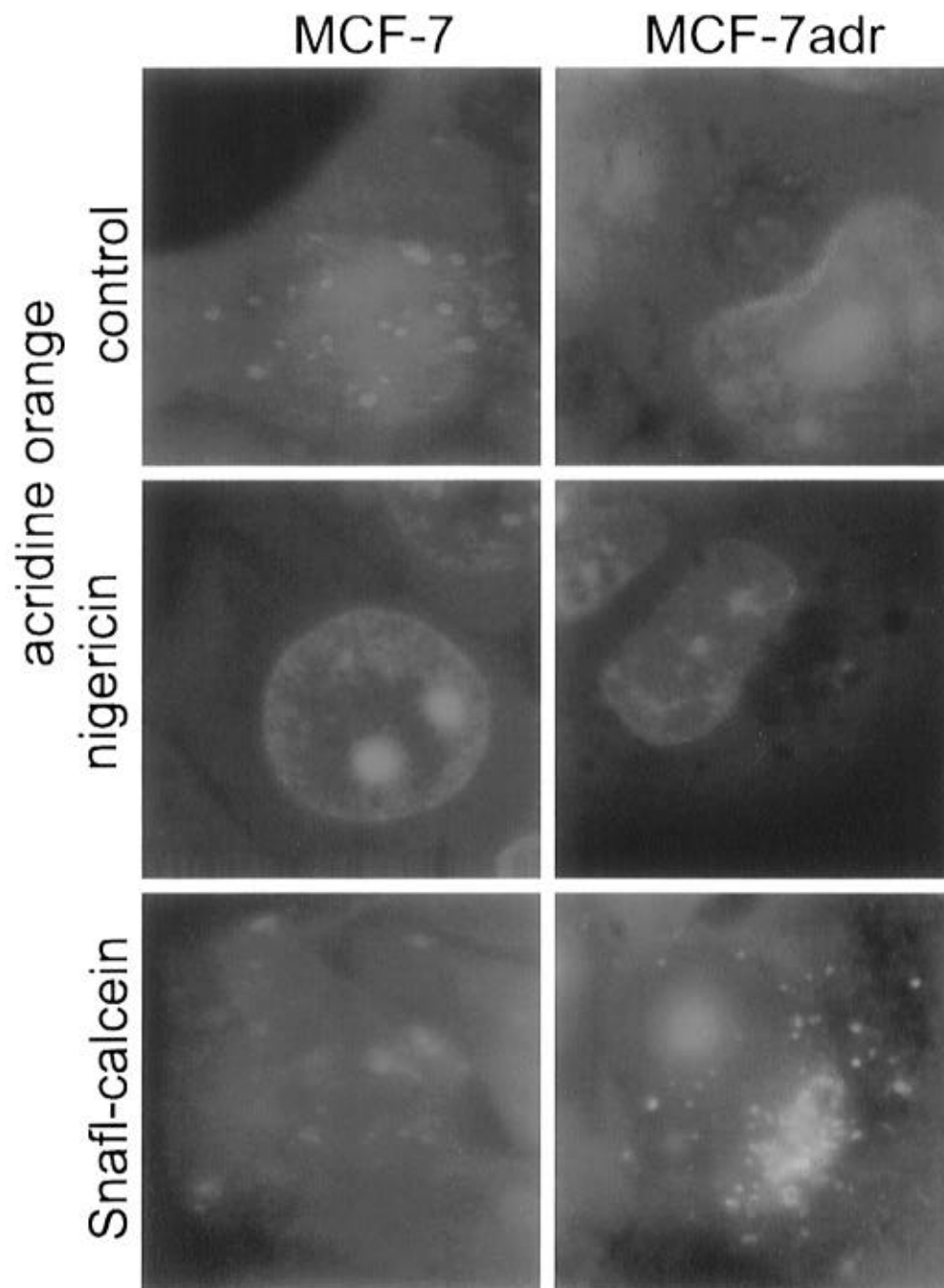


FIGURE 1: pH profiles of MCF-7 and MCF-7adr cells: labeling with acridine orange and SNAFL-calcein. Acridine orange, a label for acidic intracellular compartments, (a, top left) demonstrates a weaker and more disperse vesicular labeling for the drug-sensitive human breast cancer cells (MCF-7) in contrast to (b, top right) the labeling of the adriamycin-resistant variant (MCF-7adr). Treatment of both cell lines with nigericin ($7.5 \mu\text{M}$) at room temperature resulted in the immediate loss of orange-red fluorescence within (c, middle left) MCF-7 and (d, middle right) MCF-7adr cells. It is interesting to note that although the pericentriolar region in MCF-7adr cells no longer stains red with acridine orange following treatment with nigericin, it remains visible as an unstained area within a green fluorescent background (d). This suggests that it is the pH gradient between the pericentriolar compartment/vesicles and the cytoplasm that is altered, not its integrity, during the period of examination. Gradients of intracellular pH between the luminal compartments of intracellular vesicles and the cytoplasm are relatively absent from MCF-7 cells (e, bottom left) as assayed by the fluorescence emission of SNAFL-calcein which fluoresces green in an acidic milieu and orange-red when alkaline. In contrast, significant pH gradients are observed between vesicular lumens and the cytoplasm in MCF-7adr cells (f, bottom right). Orange-red-labeled structures in both images are mitochondria. The basic environment of the mitochondria matrix results in the red emission of the SNAFL-calcein probe.

We examined the possibility that the aberrant elevated vesicular pH observed in MCF-7 cells is reflected in altered organization and activity of these compartments. Bodipy-ceramide, a fluorescent marker for the *trans*-Golgi network and secretory vesicles, showed a dispersed tubulovesicular distribution in MCF-7 cells (Figure 3a) (Pagano et al., 1991). A large number of vesicular and cisternal structures appeared to be interconnected and possibly budding from a thin

reticular network (enlarged image in Figure 3b). It appears relevant that, for Hep-2 cells in which the cytoplasm has been experimentally acidified, the secretory vesicles also appear to be inhibited from budding from the TGN (Hansen et al., 1993). This is consistent with other reports demonstrating that an acidified cytoplasm (≤ 6.7) inhibits both endocytosis and exocytosis (Davoust et al., 1987; Cosson et al., 1989). However, in those studies it was not determined

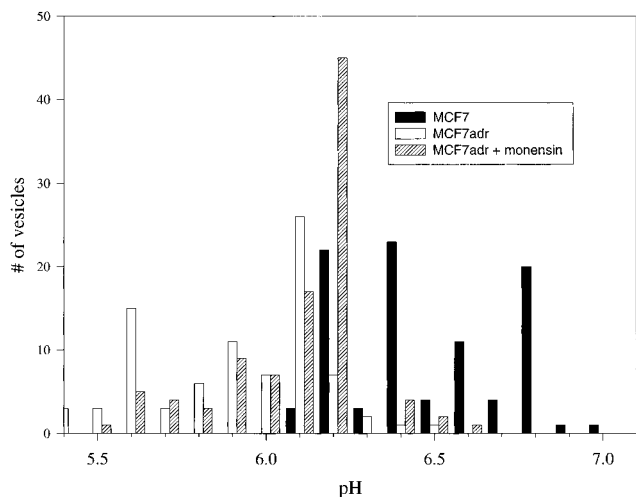


FIGURE 2: Measurements of intravesicular pH utilizing SNAFL-calcein. The vesicular pH of the drug-resistant MCF-7adr cells (white bars) is acidic with a weighted average pH of 5.9. In contrast, the vesicular pH of their drug-sensitive parental cells (black bars) is relatively neutral with a weighted average pH of 6.5. The acidic pH of the MCF-7adr cells is shifted alkaline by treatment with 2 μ M monensin (hatched bars).

whether the acidification strategies employed also caused changes in intravesicular pH. In contrast, MCF-7adr cells labeled with Bodipy-ceramide demonstrate asymmetrically localized pericentriolar structures characteristic of the *trans*-Golgi network (Figure 3c). In addition, structures identifiable as small labeled secretory vesicles (arrows) were found dispersed between the pericentriolar TGN and the plasma membrane (Figure 3d). Such vesicles were not easily detected in the MCF-7 cells (Figure 3a). The fragmented appearance of the TGN does not seem to be related to a disrupted microtubule network, as determined utilizing FITC (secondary antibody) antitubulin staining (data not shown). The organization and activity of the pericentriolar recycling compartment (Maxfield & Yamashiro, 1991) were examined with Bodipy-lactalbumin. Bodipy-lactalbumin is a marker for fluid-phase endocytosis. Following internalization, it accumulates in the pericentriolar recycling compartment prior to secretion back to the cell surface. Steady-state labeling of MCF-7 cells (Figure 3e) showed uptake of fluorescent protein into peripheral vesicular compartments. No accumulation or aggregation of fluorescent vesicles was observed in association with a compartment within the pericentriolar region of the cytoplasm. In contrast, MCF-7adr cells (Figure 3f) showed vesicles labeled with Bodipy-lactalbumin associated with a labeled pericentriolar compartment.

These observations indicate that there are two differences in the secretory pathway between the MCF-7 and MCF-7adr cells. First, the PRC is fragmented and the TGN is dispersed and localized in patches surrounding the nucleus in the drug-sensitive MCF-7 cells. Second, these two compartments, which are normally acidified in most cells, are poorly acidified in the drug-sensitive MCF-7 cells, resulting in only a modest pH gradient between the luminal compartments and the cytoplasm (Table 1). The most frequently used chemotherapeutic in breast cancer is adriamycin, which, like most chemotherapeutics, is a weak base with a pK_a of 7.8. The protonated form of adriamycin does not freely diffuse across membranes. Accordingly, adriamycin should be trapped at higher concentrations in the relatively more acidic

cytoplasm of the drug-sensitive MCF-7 cells. In contrast, adriamycin should be at lower concentrations in the more alkaline cytoplasm of the drug-resistant MCF-7adr cells. Further, any cytoplasmic adriamycin that diffuses into acidic organelles would become protonated and sequestered from the cytoplasm and nucleoplasm. Adriamycin, "ionically trapped" in the acidic compartments of the secretory pathway, would then be secreted from the cell.

This suggested that the sensitivity of drug-resistant cells to adriamycin might be overcome by chemically interfering with the capacity of these cells to acidify the vesicles that mediate internalization and secretion. Monensin is a sodium proton ionophore. It causes the pH to shift alkaline in the acidic compartments of the secretory pathway and the cytoplasm (Tartakoff, 1983a,b). As demonstrated in Figure 4, addition of 2 μ M monensin to MCF-7adr cells, in the absence of adriamycin, reduced cell viability by only 5%. However, this concentration of monensin increased the lethality of 500 nM adriamycin from 20% up to over 90%. This is close to the adriamycin sensitivity of drug-sensitive MCF-7 cells. The enhanced drug sensitivity of MCF-7adr cells treated with monensin was directly correlated with a significant decrease in the acidity of the acidic vesicular compartments to pH values observed for drug-sensitive MCF-7 cells (Figure 2).

These effects of monensin on drug sensitivity are significant in light of the recent report of only a small pH differential (0.2 pH unit) between MCF-7 and MCF-7adr cells (Altenberg et al., 1993). This difference was suggested to be too small to account for the drug resistance phenotype. Our results confirm a difference of 0.2–0.3 pH unit of total averaged cellular pH between the two cell types. However, on a subcellular level the difference in pH between the two cell types is striking: the MCF-7 cells are devoid of intracellular pH gradients (see Figure 1 and Table 1). In light of the effects on drug sensitivity of disrupting vesicular pH (see Figure 4), it appears that the pH gradient between the luminal compartments of acidic vesicles and the cytoplasm may be the more relevant parameter involved in pH regulation of drug resistance of MCF-7adr cells. These shifts of drug sensitivity did not result from disruption of the Golgi, since Brefeldin A [which breaks down the Golgi but does not disrupt transport from the *trans*-Golgi or PRC to the cell surface (Lippincott-Schwartz et al., 1991)] did not affect drug sensitivity (data not shown). Although each of the drugs might also affect the pH of the lysosomes, this organelle is unlikely to play a significant role in affecting the sensitivity of the cells to chemotherapeutics. The drugs would be expected to be rapidly protonated and sequestered within these organelles. However, an equilibrium would quickly be reached between the cytoplasm and lysosomal compartment, limiting the utility of a relatively static compartment in effluxing drug. In contrast, the PRC and TGN are actively turning over their luminal contents to the cell surface (Wieland et al., 1987), providing a continuous efflux pathway for adriamycin.

Organelle acidification affects intracellular targeting, e.g., fusions of endosomes, secretory vesicles, and lysosomes; uncoupling of ligands from membrane receptors; processing and degradation of proteins; targeting of lysosomal enzymes; and glycosylation and packaging of secretory glycoproteins/glycolipids (Mellman et al., 1986; Maxfield & Yamashiro, 1991; van Deurs et al., 1989). This communication dem-

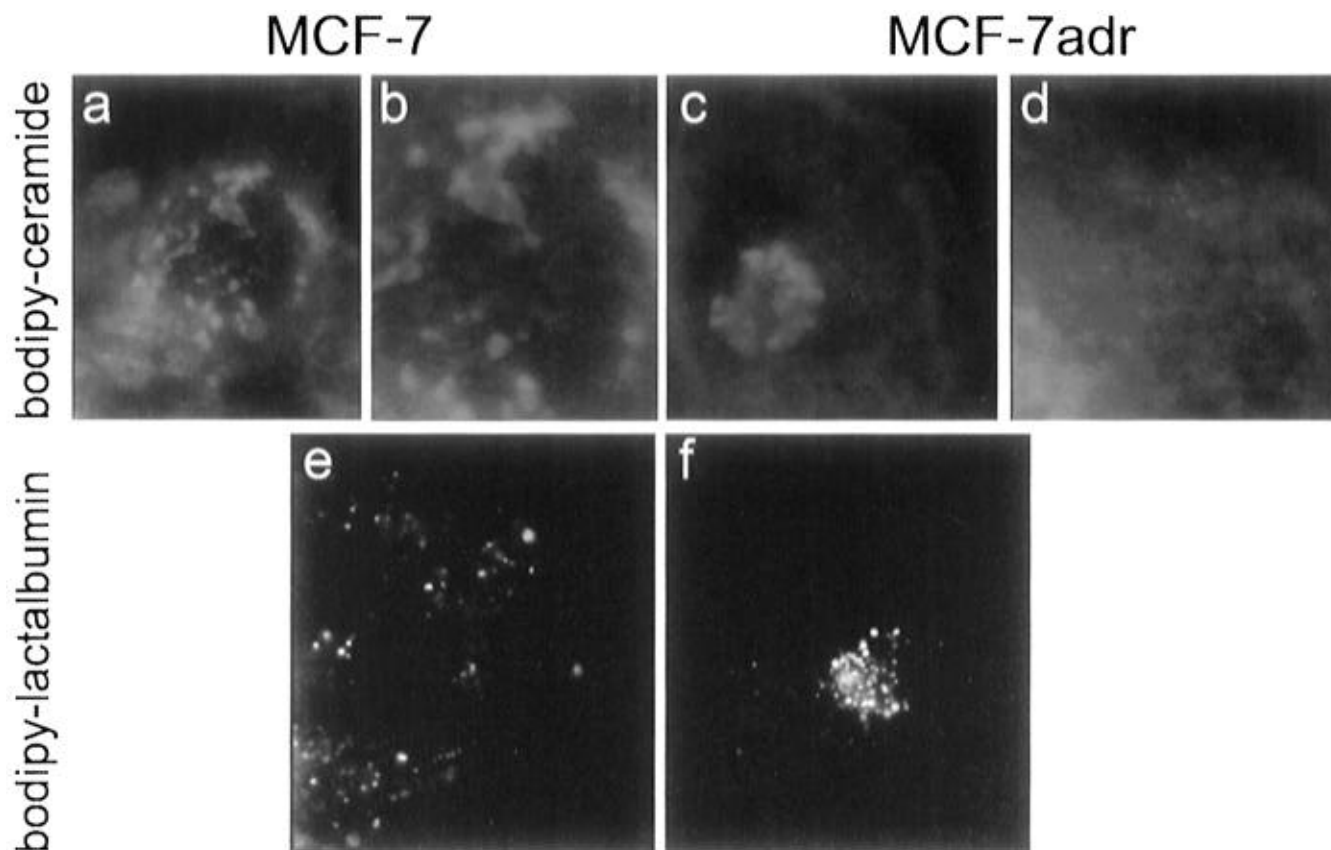


FIGURE 3: Fluorescent labeling of the exocytotic vesicles: TGN and secretory vesicles with Bodipy-ceramide and PRC with Bodipy-lactalbumin. (a) The *trans*-Golgi compartments of MCF-7 cells (orange-labeled structures) are diffusely localized throughout the cytoplasm and, as observed in the enlargement (b), are organized as a collection of vesicular and cisternal structures, interconnected via fine tubules. (c) In contrast, the *trans*-Golgi compartments of MCF-7adr cells are compact and pericentriolar, and as observed in the enlargement (d), there are small secretory vesicles (orange-labeled vesicles) in the cytosol. (e) The diffuse distribution of endocytosed vesicles containing internalized Bodipy-lactalbumin (white vesicles) in MCF-7 cells is different from the (f) compact pericentriolar localization observed in the drug-resistant MCF-7adr cells.

onstrates that the human breast cancer cell line (MCF-7) is defective in both the acidification of intracellular vesicles (Figure 1) and the organization of the PRC and the TGN (Figure 3). The relationship between the fragmentation of the TGN and PRC and the aberrant acidification of tubulovesicular structures comprising these structures remains to be determined. Staining cells with fluorescently labeled antitubulin suggests that this fragmentation (TGN) and disruption (PRC) are not a consequence of disorganization of the microtubule network, although treatment with nocodazole can phenotypically mimic these disruptions in structure (data not shown). These abnormalities in both vesicular pH and organellar structure appear repaired in the adriamycin-resistant variant of this cell line (MCF-7adr). The role of a collapsed transvesicular pH gradient as the primary factor in producing drug sensitivity was strongly supported by results with ionophores. Monensin and nigericin enhanced the drug sensitivity of adriamycin-resistant cells (MCF-7adr).

A mechanism for the pH shift observed in this study is suggested from work on parallel systems that show a similar shift in the luminal pH of intracellular organelles. In endocytotic and secretory compartments, an electrogenic ATPase coupled to a Cl^- conductance is responsible for maintaining the low pH (Al-Awqati et al., 1992; Barasch et al., 1991; Van Dyke & Belcher, 1994). In cystic fibrosis, a decreased chloride conductance in the *trans*-Golgi compartment and recycling endosomes causes an alkaline shift of

organellar pH (Al-Awqati et al., 1992; Barasch et al., 1991). In the absence of chloride, the luminal pH shifts 0.4–0.6 unit alkaline in multivesicular bodies (MVB), CURL vesicles, and receptor recycling compartments (Van Dyke & Belcher, 1994). The comparison with cystic fibrosis may be relevant in that the cystic fibrosis transmembrane regulator (CFTR) is involved in Cl^- conductance, aberrant in cystic fibrosis, and has been demonstrated to be homologous to the P-glycoprotein. The P-glycoprotein, which is expressed in MCF-7adr cells and involved in producing the drug resistance phenotype (Simon & Schindler, 1994) has also been reported to function as a Cl^- channel (Valverde et al., 1992) or modify chloride conductance [Hardy et al., 1995; Luckie et al., 1994; but see also Ehring et al. (1994)]. Like the CFTR, the P-glycoprotein is also observed in the Golgi, vesicular, and plasma membranes (Willingham et al., 1987; Molinari et al., 1994).

The relationship between CFTR and P-glycoprotein suggests that an aberrant chloride conductance in the organelles of MCF-7 cells may similarly cause the organelle pH to shift alkaline (see Table 1). Likewise, the activation of a chloride conductance, or expression of a Cl^- ion channel, in the MCF-7adr cells may then normalize the pH within acidic compartments. Adriamycin and a large number of drugs utilized for chemotherapy are weak bases which can be protonated and, thus, trapped in acidic compartments. Drug sensitivity of MCF-7 cells may be a consequence of an inability to protonate, sequester, and then secrete these drugs (PSS

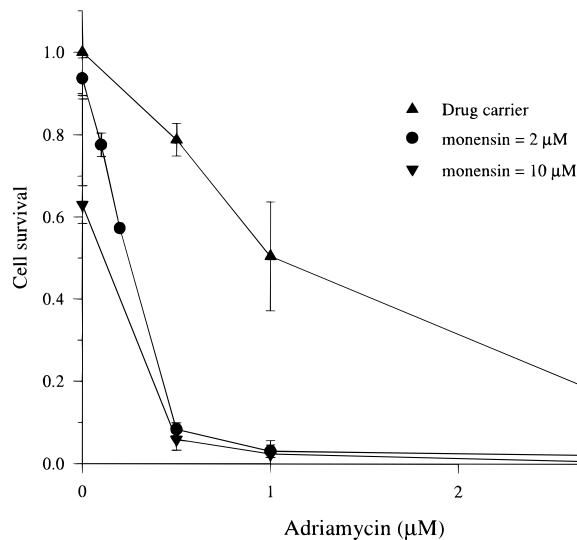


FIGURE 4: Adriamycin sensitivity of MCF-7adr cells in the absence and presence of monensin. Drugs that dissipate the pH gradient across the Golgi significantly increase the sensitivity of drug-resistant MCF-7adr cells to adriamycin. Cells were exposed to the indicated dosage of adriamycin for 6 h in the presence of 0, 2, or 10 μM monensin. The cells were then washed and grown in media without adriamycin and without monensin for 3 days. The number of surviving cells was plotted with a value of 1.0 representing the maximum number of cells which was obtained in the absence of either adriamycin or monensin.

model). Drug resistance is then an “ionic rehabilitation” of the normally acidic intracellular compartments through the expression of proteins (e.g., chloride channels or proton pumps) that compensate for this defect in acidification within tumor cells.

While multidrug resistance is likely to be the consequence of diverse mechanisms (Simon & Schindler, 1994), the ability to reverse drug resistance by drugs that alkalinize the pH in acidic compartments of the endosomal and secretory systems indicates that protonation, sequestration, and secretion are the principle elements of the primary mechanism for drug resistance in the MCF-7 breast cancer line. Any manipulations that either affect acidification or transport through these organelles should affect drug sensitivity. It is possible that the Golgi, particularly the secretory compartments, may normally play a role in protecting all cells from environmental toxins that are weak bases.

REFERENCES

- Al-Awqati, Q., Barasch, J., & Landry, D. (1992) *J. Exp. Biol.* 172, 245.
- Altenberg, G. A., Young, G., Horton, J. K., Glass, D., Belli, J. A., & Reuss, L. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90, 9735.
- Barasch, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D., & Al-Awqati, Q. (1991) *Nature (London)* 352, 70.

- Beck, W. T. (1987) *Biochem. Pharmacol.* 36, 2879.
- Cosson, P., De Curtis, I., Pouyssegur, J., Griffiths, G., & Davoust, J. (1989) *J. Cell Biol.* 108, 377.
- Dalmark, M., & Hoffmann, E. K. (1983) *Scand. J. Clin. Lab. Invest.* 43, 241.
- Davoust, J., Gruenberg, J., & Howell, K. E. (1987) *EMBO J.* 6, 3601.
- Ehring, G. R., Osipchuk, Y. V., & Cahalan, M. D. (1994) *J. Gen. Physiol.* 104, 1129.
- Hansen, S. H., Sandvig, K., & van Deurs, B. (1993) *J. Cell Biol.* 121, 61.
- Hardy, S. P., Goodfellow, H. R., Valverde, M. A., Gill, D. R., Sepúlveda, F. V., & Higgins, C. F. (1995) *EMBO J.* 14, 68.
- Lippincott-Schwartz, J., Yuan, L. C., Tipper, C., Amherdt, M., Orci, L., & Klausner, R. D. (1991) *Cell* 67, 601.
- Luckie, D. B., Krouse, M. E., Harper, K. L., Law, T. C., & Wine, J. J. (1994) *Am. J. Physiol. Cell Physiol.* 267, C650.
- Maxfield, F. R., & Yamashiro, D. J. (1991) in *Intracellular trafficking of proteins* (Steer, C. J., & Hanover, J. A., Eds.) pp 157–182, Cambridge University Press, Cambridge.
- McGraw, T. E., Dunn, K. W., & Maxfield, F. R. (1993) *J. Cell Physiol.* 155, 579.
- Mellman, I., Fuchs, R., & Helenius, A. (1986) *Annu. Rev. Biochem.* 55, 663.
- Molinari, A., Cianfriglia, M., Meschini, S., Calcabrini, A., & Arancia, G. (1994) *Int. J. Cancer* 59, 789.
- Pagano, R. E., Sepanski, M. A., & Martin, O. C. (1989) *J. Cell Biol.* 109, 2067.
- Pagano, R. E., Martin, O. C., Kang, H. C., & Haugland, R. P. (1991) *J. Cell Biol.* 113, 1267.
- Presley, J. F., Mayor, S., Dunn, K. W., Johnson, L. S., McGraw, T. E., & Maxfield, F. R. (1993) *J. Cell Biol.* 122, 1231.
- Roepe, P. D., Weisburg, J. H., Luz, J. G., Hoffman, M. M., & Wei, L.-Y. (1994) *Biochemistry* 33, 11008.
- Russell, J. T., & Holz, R. W. (1981) *J. Biol. Chem.* 256, 5950.
- Sehested, M., Skovsgaard, T., & Roed, H. (1988) *Biochem. Pharmacol.* 37, 3305.
- Simon, S. M., & Schindler, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 3497.
- Simon, S. M., Roy, D., & Schindler, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 1128.
- Tartakoff, A. M. (1983a) *Methods Enzymol.* 98, 47.
- Tartakoff, A. M. (1983b) *Cell* 32, 1026.
- Valverde, M. A., Díaz, M., Sepúlveda, F. V., Gill, D. R., Hyde, S. C., & Higgins, C.F. (1992) *Nature (London)* 355, 830.
- van Deurs, B., Petersen, O. W., Olsnes, S., & Sandvig, K. (1989) *Int. Rev. Cytol.* 117, 131.
- Van Dyke, R., & Belcher, J. D. (1994) *Am. J. Physiol. Cell Physiol.* 266, C81.
- Warburg, O. (1956) *Science* 123, 309.
- Whitaker, J. E., Haugland, R. P., & Prendergast, F. G. (1991) *Anal. Biochem.* 194, 330.
- Wieland, F. T., Gleason, M. L., Serafini, T. A., & Rothman, J. E. (1987) *Cell* 50, 289.
- Willingham, M. C., Richert, N. D., Cornwell, M. M., Tsuruo, T., Hamada, H., Gottesman, M. M., & Pastan, I. H. (1987) *J. Histochem. Cytochem.* 35, 1451.

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