Int J Biochem Mol Biol 2011;2(3):219-227
www.ijbmb.org /ISSN:2152-4114/IJBMB1104008

Original Article

Altered intracellular pH regulation in cells with high levels of P-glycoprotein expression

Gregory Young, Luis Reuss, Guillermo A. Altenberg

Department of Cell Physiology and Molecular Biophysics, and Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock, Texas, USA

Received April 27, 2011; accepted May 4, 2011; Epub June 3, 2010; Published September 30, 2011

Abstract: P-glycoprotein is an ATP-binding-cassette transporter that pumps many structurally unrelated drugs out of cells through an ATP-dependent mechanism. As a result, multidrug-resistant cells that overexpress P-glycoprotein have reduced intracellular steady-state levels of a variety of chemotherapeutic agents. In addition, increased cytosolic pH has been a frequent finding in multidrug-resistant cells that express P-glycoprotein, and it has been proposed that this consequence of P-glycoprotein expression may contribute to the lower intracellular levels of chemotherapeutic agents. In these studies, we measured intracellular pH and the rate of acid extrusion in response to an acid load in two cells with very different levels of P-glycoprotein expression: V79 parental cells and LZ-8 multidrug resistant cells. Compared to the wild-type V79 cells, LZ-8 cells have a lower intracellular pH and a slower recovery of intracellular pH after an acid load. The data also show that LZ-8 cells have reduced ability to extrude acid, probably due to a decrease in Na^+/H^+ exchanger activity. The alterations in intracellular pH and acid extrusion in LZ-8 cells are reversed by 24-h exposure to the multidrug-resistance modulator verapamil. The lower intracellular pH in LZ-8 indicates that intracellular alkalinization is not necessary for multidrug resistance. The reversal by verapamil of the decreased acid-extrusion suggests that P-glycoprotein can affect other membrane transport mechanism.

Keywords: MDR1, Na^+/H^+ exchanger, verapamil, multidrug resistance, Adriamycin, intracellular pH regulation

Introduction

Overexpression of P-glycoprotein (Pgp, MDR1, ABCB1), a 170-kD plasma membrane glycoprotein, produces multidrug resistance in tumor cells and mammalian cells in culture [1,2]. Multidrug resistance is characterized by resistance to the cytotoxic effects of a wide variety of chemically unrelated drugs that have different mechanisms of action [1-3]. Multidrug-resistant cells that overexpress Pgp have reduced intracellular steady-state levels of a variety of chemotherapeutic agents, which results primarily from pumping of the drugs out of the cells by Pgp [1-3].

Increased intracellular pH (pH_i) has been a frequent finding in Pgp-expressing multidrug-resistant cells [4-12], and it has been proposed that this alteration is a consequence of Pgp expression that contributes to the reduced intracellular levels of chemotherapeutic agents [4,7,9,10,12]. Alterations in pH_i in multidrug-resistant cells are potentially important because most Pgp substrates are liposoluble, aromatic, and positively charged at physiologic extracellular pH [13,14]. It is likely that the concentration of uncharged (lipophilic) moiety of the drug is similar on both sides of the plasma membrane; the pH_i level would then determine the concentration of the protonated (slowly-permeant) moiety, and thus its total intracellular concentration.

Although alterations of pH_i can result from changes in the activity of pH_i regulatory mechanisms, there are very few studies of these mechanisms in Pgp-expressing cells. In two studies an increase in the Na^+-dependent rate of pH_i recovery was reported [5,11]. However, the intracellular buffering power was not determined and hence it is possible that the increase in pH_i recovery rate is the result of reduced cytosolic H^+ buffering, and not increased efflux of H^+ equivalents (J_H). In view of the possible role of pH_i in multidrug resistance and the absence of

measurements of J_H during pH_i recovery from an acid load, we decided to further study pH_i and its regulation in two Chinese hamster fibroblast cell lines with very different Pgp levels.

Materials and methods

Cells and Western blots

Experiments were performed on subconfluent monolayers of wild-type (V79) and multidrug-resistant (LZ-8) Chinese hamster lung fibroblasts. Cell lines were grown in F10 medium supplemented with 10% FBS, with the addition of Adriamycin in the case of LZ-8 cells. Adriamycin, a fluorescent drug, was removed from the culture medium 1 day prior to the experiments. Both cell lines express Pgp, but its level at the plasma membrane of LZ-8 cells is >20-fold that of V79 cells [15]. This translates in increased resistance to Adriamycin, vinblastine and colchicine (~3,000-, 200-, and 300-fold, respectively, compared to V79 cells), and faster rate of efflux of Adriamycin and rhodamine 123 [15-18]. Additional details on these cell lines have been published [15-17]. Inmunoblots were performed on crude membranes subjected to 7% SDS-PAGE using the anti-P-glycoprotein antibody C219 (Covance, Princeton, NJ). Detection was by enhanced chemiluminescence (ECL, GE Healthcare).

Experimental setup and solutions

Cells were plated in 60-160 mm-thick, 25-mm-diameter round coverslips in a solution (control solution) of the following composition: 25 mM HCO_3^-, 115 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1.5 mM Na phosphate, and 8.3 mM glucose, equilibrated with 95% O_2/5% CO_2, pH 7.42-7.43. The Hepes-buffered solution used to determine intrinsic buffering power (β_i) contained: 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$ 8.3 mM glucose, and 10 mM Hepes/NaOH, pH 7.42-7.43 (air equilibrated). When used, NH_4Cl partially replaced NaCl mol-by-mol. Nigericin was used at a concentration of 5μM, from a 10 mM stock in ethanol. Amiloride (1 mM) was dissolved directly in the experimental solutions and verapamil (25 μM) was added from a 5 mM stock in water.

pH_i measurements

pH_i was measured using the pH-sensitive fluorescent probe 2'7'-biscarboxyethly-5(6) carboxyfluorescein (BCECF), essentially as described [16]. Cells were loaded with the pH-sensitive probe by a 60-min incubation with 10 μM tetraacetoxymethyl ester of BCECF (BCECF-AM), at 37°C. After loading, the cells were immediately used or kept for up to 1 h before use in Petri dishes with control solution without BCECF-AM. The degree of BCECF loading was not statistically different in V79 and LZ-8 cells, and the BCECF leakage from both cell lines was similar (~0.3%/min). The coverslips with the BCECF-loaded cells were mounted on a chamber (Leiden microincubator, Medical Systems Corp., Greenvalle, NY) placed on the stage of an inverted microscope (Nikon Diaphot, Nikon, Tokyo). The cells were superfused by gravity at a rate of 15-25 ml/min, at 37°C, using a system based on glass condensers. The pH and temperature of the solution bathing the cells was indistinguishable from that of the control solution at 37°C, indicating no significant heat or CO_2 losses.

The pH_i was estimated from the BCECF fluorescence emission ratio (measured at 535 nm) at excitation wavelengths of 495 and 440 nm (F_{495}/F_{440}). Excitation light originated from a 150-W Xenon lamp was split (beam splitter, Oriel Instruments) into two parallel pathways after passing through a heat filter (Oriel Instruments, Stratford, CT). Each of the parallel pathways had a band-pass filter (440DF10 or 495DF10, Omega Optical Inc., Brattleboro, VT) and a computer-controlled shutter (Vincent Associates, Rochester, NY) in series. The shutters opened sequentially for 100 ms during each measurement to minimize photobleaching. Light from the two pathways was merged and directed to the epifluorescence attachment (Nikon) of the inverted microscope using a bifurcated optic fiber (Oriel Instruments). Illumination was restricted to the cells under study using a field diaphragm. The excitation light reflected by a dichroic mirror (515DRLP, Omega Optical Inc) was focused onto the cells with a 40X, 1.3 NA oil-immersion objective (Nikon 78820). Emitted light collected by the same objective, passed through the dichroic mirror, and then a band-pass filter (535DF35, Omega Optical Inc). A lens (CCTV adapter with 1X relay lens, Nikon) was used to defocus the fluorescent light which was measured with an end-on photomultiplier tube (77346, Oriel Instruments). Currents generated by the photomultiplier were

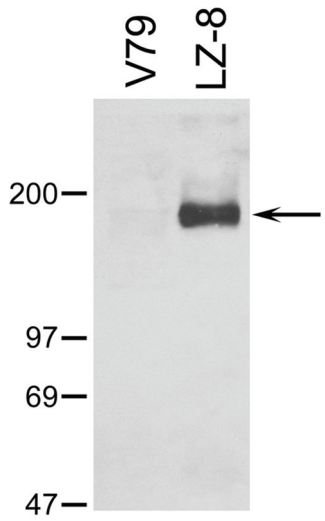

Figure 1. Expression of P-glycoprotein in membranes of V79 and LZ-8 cells. Immunoblot of crude membranes from V79 (150 μg) and LZ-8 (12.5 μg) cells probed with the anti-Pgp antibody C219. The arrow on the right points to Pgp and the molecular weight marker positions in kDa are indicated on the left side.

converted to voltages with a current preamplifier (70710, Oriel Instruments) and input into an A/D converter (TL1-DMA interface, Molecular Devices, Sunnyvale, CA). Data acquisition and shutters control were done with "in-house" software, and data analysis with SigmaPlot (Systat software, San Jose, CA).

The average fluorescence of 100-150 cells was collected, but in preliminary experiments we found similar results from single-cell recordings. Autofluorescence was less than 1% of the signal in BCECF-loaded cells and no attempt was made to correct for it. Under the loading conditions employed, there was no evidence of BCECF compartmentalization, from confocal fluorescence images, and by the lack of differences in the F_{495}/F_{440} emission ratio in different spots of single cells. In addition, 50 mM digitonin released >95% of BCECF from V79 and LZ-8 cells. Calculations of pH_i were based on the nigericin technique [19], using high-K^+ solutions containing: 10 mM KCl, 1 mM $MgCl_2$ 2 mM $CaCl_2$, 8.3 mM glucose, 20 mM NaCl, 90 mM potassium gluconate, and 10 mM Hepes, titrated to various pH values. At the end of each experiment, cells were exposed sequentially to two calibration solutions of pH 7.0 and 7.3, respectively. The F_{495}/F_{440} nm emission ratio in these solutions was normalized to the value obtained for the same pH in the average calibration curve (**Figure 2B**, see Results), and all ratios in the experiment were converted to pH using the calibration curve.

Net fluxes of acid equivalents (J_H)

These were calculated from the total intracellular buffering power (β_T) and the rates of change in pH_i according to: $J_H = (\Delta pH_i/\Delta t)\ \beta_t$. The cells b_T is the sum of the intrinsic buffering power in the nominal absence of HCO_3^-/CO_2 and the HCO_3^-/CO_2 buffering power ($\beta_T = \beta_i + \beta_{Bic}$; $\beta_{Bic} = 2.3\ \alpha\ pCO_2\ 10^{pHi-pK}$ [20], where α is the CO_2 solubility coefficient. Determination of β_i was done from the pH_i changes produced by addition of NH_4Cl at constant extracellular pH, with pH_i regulatory mechanisms blocked (Hepes-buffered solution with 1 mM amiloride, see Results). The β_i calculations are based on the assumptions that: a) NH_3 permeability is high enough to make steady-state $[NH_3]$ equal inside and outside the cells, b) NH_3 permeability >>> NH_4^+ permeability, and c) the apparent pK (pKa = 8.9) of the NH_3/NH_4^+ system at 37°C is the same inside and outside the cells. Then, β_i is calculated from $\beta_i = \Delta[NH_4^+]_I\ /\Delta pH_i$, where intracellular $[NH_4^+]$ ($[NH_4^+]_i$) is calculated from $[NH_4^+]_i = [NH_3]_i \cdot 10^{pk-pHi}$ [20].

Data presentation and statistics

Data are presented as means ± SEM. Statistical comparisons were done by Student t-tests for paired or unpaired data as appropriate, and differences were considered significant when $P < 0.05$.

Results

Figure 1 shows that the level of Pgp expression in LZ-8 cells is much higher than that in the parental V79 cells. Calibration of BCECF inside V79 and LZ-8 cells was performed by the technique of Thomas et al. [19]. **Figure 2A** illustrates the changes in the fluorescence ratio, F_{495}/F_{440}, as a function of extracellular pH in LZ-8 cells. The cells were bathed with Hepes-buffered solution, which was replaced with high-K^+ calibration solutions of the pH indicated in the top bar, in the presence of the K^+/H^+ exchanger ionophore nigericin. The first solution substitution produced a change in F_{495}/F_{440} that reached a stable value after ~1.5 min, but subsequent solu-

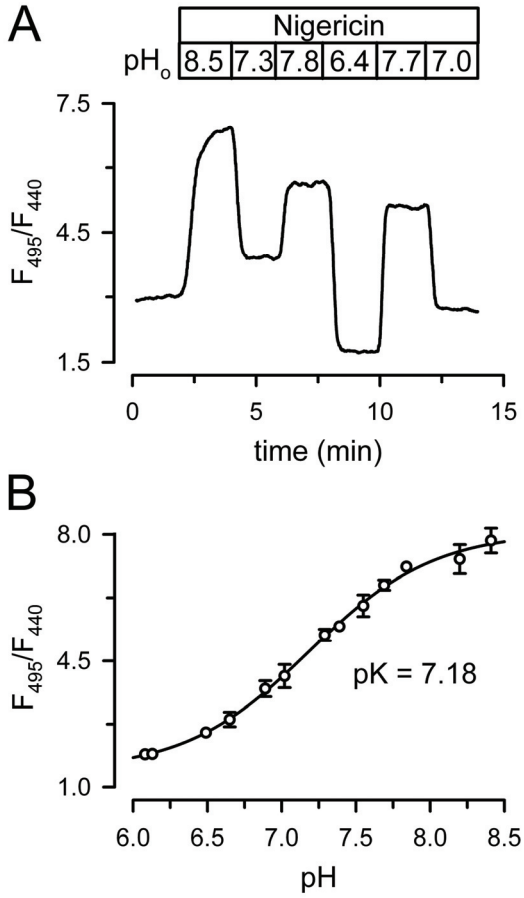

Figure 2. Intracellular calibration of the pH-sensitive probe BCECF. (**A**) Typical record showing the changes in BCECF fluorescence emission in response to changes in extracellular pH (pH_o, indicated at the top bar) in high-K^+ solutions in the presence of 5 μM nigericin. The trace displays the ratio of BCECF emission at excitation wavelengths of 495 and 440 (F_{495}/F_{440}) as a function of time. The trace shown is from LZ-8 cells. (**B**) Average F_{495}/F_{440} changes from V79 and LZ-8 pooled data (n = 10 for each cell type). Data from the two cell lines were statistically undistinguishable.

tion changes caused much faster alterations. The slow initial change probably reflects the time required for incorporation of enough nigericin into the plasma membrane to rapidly equilibrate extracellular and intracellular pH. The responses of V79 and LZ-8 cells were indistinguishable, and the pooled data were used to calculate the pKa of BCECF inside the cells (**Figure 2B**).

As shown in **Figure 3**, the calculated steady-

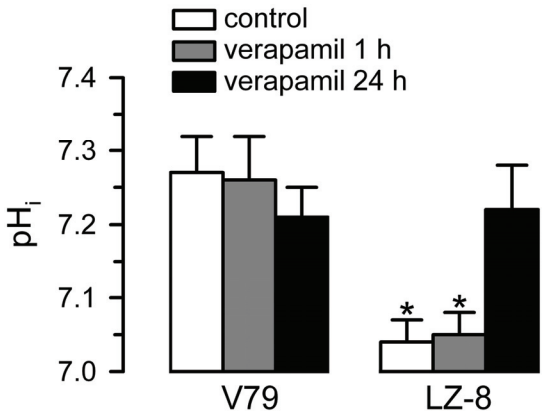

Figure 3. Basal intracellular pH (pH_i) and effects of verapamil. Data are means ± SEM from V79 (n = 16) and LZ-8 (n = 17) cells in the absence of verapamil, and exposed to verapamil for 1 h (V79, n = 6; LZ-8, n = 6), or 24 h (V79, n = 10; LZ-8, n = 8). The asterisks denote $P < 0.05$ compared to V79 cells in the absence of verapamil. Verapamil was used at a concentration of 25 μM.

state pH_i in HCO_3^-/CO_2-buffered solution was lower in LZ-8 than in V79 cells. Exposure to 25 μM of verapamil for 1 h did not affect pH_i in either cell line (**Figure 3**). However, 24-h exposure to verapamil (in the culture medium and all experimental solutions) elevated LZ-8 cells pH_i to a value statistically indistinguishable from that in V79 cells (**Figure 3**). Verapamil is a modulator of multidrug resistance that blocks transport of other Pgp substrates; at 25 μM, it increases the cytotoxic effects of Adriamycin and vinblastine, and rapidly reduces the unidirectional efflux of rhodamine 123 [18,21,22].

The efficiency of the cells to recover from an acid load was studied following the changes in pH_i produced by transient exposure to 25 mM NH_4Cl (**Figure 4**) [16,20]. Upon addition of NH_4Cl, pH_i increases due to rapid NH_3 entry which buffers H^+, generating NH_4^+ and raising pH_i. After the peak alkalinization, there is a slow pH_i decrease ("plateau acidification") attributed to influx of H^+ equivalents in the form of NH_4^+, which produces the acid load. Upon NH_4Cl removal, pH_i falls rapidly to a minimum below control because of the rapid NH_3 efflux and the intracellular trapping of H^+ from the dissociation of NH_4^+. Then, pH_i returns to control values by the action of membrane transport mechanisms that extrude H^+ equivalents. As shown in **Figure 4**, pH_i recovery is faster in V79 (**Figure 4A**) than

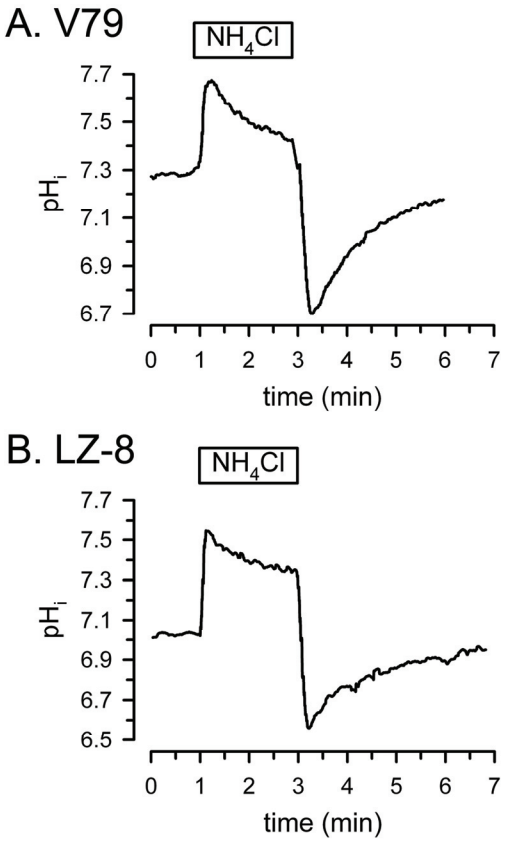

Figure 4. Recovery of pH_i from an acid load. Response of V79 (**A**) and LZ-8 (**B**) cells to an acid load elicited by transient exposure to 25 mM NH_4Cl.

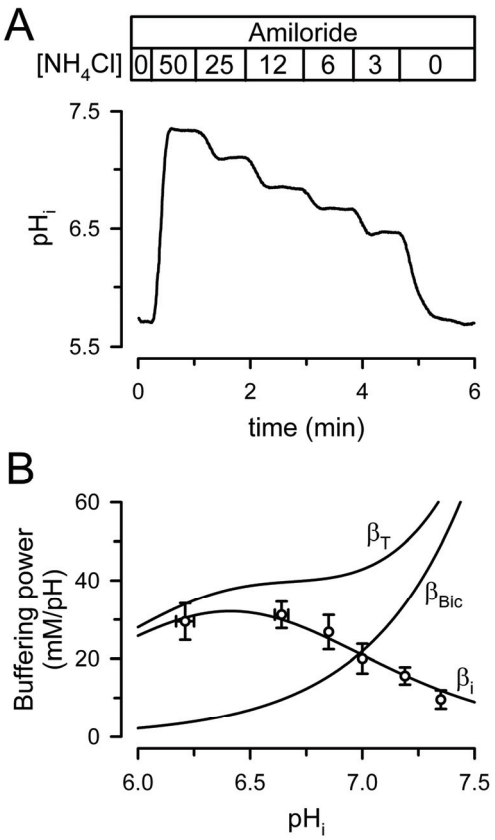

Figure 5. Intracellular buffering power. (**A**) Typical record showing the changes in pH_i in response to changes in bath solution [NH_4Cl] (indicated in the top bar, in mM) in cells superfused with Hepes-buffered solution in the presence of 1 mM amiloride. Under these conditions intracellular pH_i regulation is abolished and the pH_i changes can be used to calculate the intrinsic buffering power (β_i, see Materials and methods for details). The trace shown was obtained in V79 cells. (**B**) Average β_i from V79 (n = 9) and LZ-8 (n = 10) pooled data. Data from the two cell lines were statistically indistinguishable, and are presented as open circles (means ± SEM). SEMs smaller than the symbols are not shown. The solid line labeled β_i is the fit of the data to: $\beta_i = 2.3 \cdot K \cdot [IB] \cdot [H^+]/(K + [H^+])^2$ [see 20] where K and [IB] are the intracellular buffers dissociation constant and concentration, respectively. The estimates of [IB] and pK were 56 mM and 6.41, respectively. The β_{Bic} line is the HCO_3^-/CO_2 buffering (see Materials and methods) and β_T is the sum of β_i and β_T.

in LZ-8 cells (**Figure 4B**).

Although the basal pH_i was lower in LZ-8 cells, pH_i was similar in both cell lines immediately after removal of NH_4Cl (V79 = 6.62 ± 0.11, n = 20; LZ-8 = 6.52 ± 0.13, n = 16, P > 0.05) indicating that the acid loading procedure was more efficient in V79 cells. This is expressed by the slower acidification during exposure to NH_4Cl in LZ-8 cells (**Figure 4**; V79 ΔpH_i = 0.40 ± 0.06, n = 20; ΔpH_i LZ-8 = 0.21 ± 0.05, n = 16; P < 0.05). This alteration was reversed by 24-h exposure of LZ-8 cells to 10 μM verapamil (data not shown).

The reduced pH_i recovery from an acid load in LZ-8 cells can result from a decrease in the activity of pH_i regulatory mechanisms (*i.e.*, real fall in J_H), and/or to an elevation of the intracellular buffering power (same J_H, with slower pH_i change due to increased buffering). To distinguish between these possibilities, β_i was measured as detailed in "Materials and methods", and exemplified in **Figure 5A**. The cell shown was acid-loaded by exposure to 50 mM NH_4Cl in Hepes-buffered solution with 1 mM amiloride added at the time of NH_4Cl removal. At 1 mM,

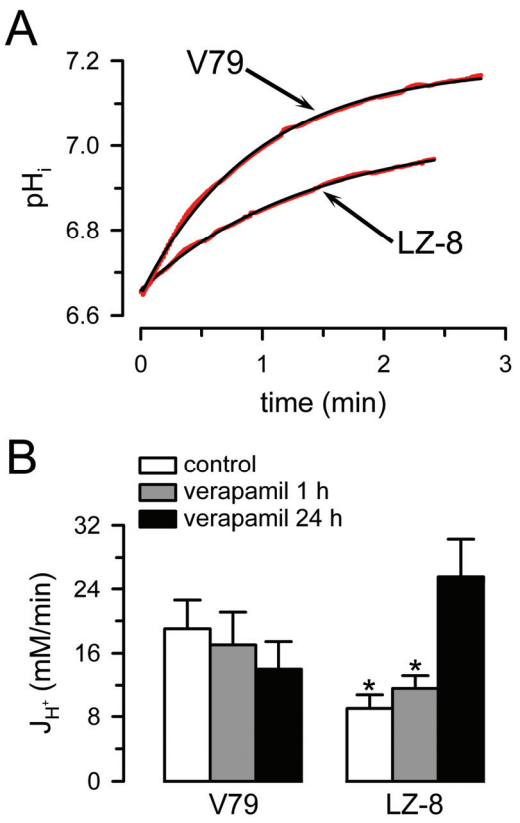

Figure 6. Efflux of H^+ equivalents after an acid load. (**A**) Average traces of pH_i recovery after an acid load (see Figure 4). The records shown in red are averages from n = 8 experiments in each of the cell lines. The black lines are single-exponential fits to the data that yielded rate constants of 1.05 and 0.73 min^{-1} for the V79 and LZ-8 data, respectively. (**B**) Average efflux of H^+ equivalents (J_H) after the acid load. The J_H values were calculated from the $\Delta pH_i/\Delta time$ data and the intracellular buffering power (β_T), and are shown as means ± SEM. Data are from V79 (n = 20) and LZ-8 (n = 13) cells in the absence of verapamil, and exposed to verapamil for 1 h (V79, n = 6; LZ-8, n = 6), or 24 h (V79, n = 6; LZ-8, n = 7). The asterisks denote $P < 0.05$ compared to V79 cells in the absence of verapamil. Verapamil was used at a concentration of 25 μM.

amiloride completely blocks pH_i regulation (note that pH_i is the same at the beginning and end of the record). The pH_i changes were measured upon exposure to several NH_4Cl concentrations, and β_i was calculated from the changes in pH_i and $[NH_4^+]$ (see "Materials and methods"). The values of β_i obtained in V79 and LZ-8 did not differ significantly, and the pooled data are shown in **Figure 5B**. **Figure 5B** also shows β_{Bic} and β_T, calculated as described in "Materials and methods" [20]. From the analysis of the data in **Figure 3** and **Figure 5**, under control conditions, β_T is significantly higher in V79 than in LZ-8 cells, and HCO_3^-/CO_2 is the main buffer system in these cells (~75%), whereas it contributes less (~50%) to β_T in LZ-8 cells. The reason for these differences is the lower control pH_i in LZ-8 compared to V79 cells since β_T and its dependency on pH_i were similar in both cells lines. Since the minimum pH_i after NH_4Cl removal (see above) and β_T (**Figure 5B**) were the same in V79 and LZ-8 cells, the slower pH_i recovery in LZ-8 cells (**Figure 5A**) can be unambiguously attributed to a decreased activity of pH_i recovery mechanisms. **Figure 6** shows average pH_i recovery data from V79 and LZ-8 cells (**Figure 6A**), as well as the calculated initial rates of J_H (**Figure 6B**). Since the rates of pH_i recovery follow single exponential rises to the control pH_i, and β_T changes between pH_i of 6.5 and 7.2 are relatively minor, it follows that J_H is faster in V79 than in LZ-8 cells in the whole pH_i range studied.

Exposure to verapamil for 1 h did not affect pH_i recovery from the acid load, but 24-hour exposure to verapamil enhanced the ability of LZ-8 cells to recover from an acid load to values comparable to those of V79 cells (**Figure 6B**). The effect of verapamil on β_t was small or nil, as indicated by similar changes in pH_i upon addition and removal of NH_4Cl in V79 and LZ-8 cells treated with verapamil, compared to changes in the absence of the drug (data not shown).

Discussion

The results of the present experiments show that, compared to the wild-type V79 cells, the highly multidrug-resistant LZ-8 cells have a lower pH_i, a slower acidification during exposure of NH_4Cl, and a slower pH_i recovery upon removal of NH_4Cl.

The reduced acidification during exposure to NH_4Cl in LZ-8 *vs*. V79 cells can be the result of a slower rate of NH_4^+ influx, and/or a decrease in activity of pH_i regulatory mechanisms operative during the intracellular alkalinization produced by NH_3 entry. Experiments to discriminate among these possibilities were not performed. However, a reduced NH_4^+ seems likely because NH_4^+ influx into the cells may occur *via* the Na^+/H^+ exchanger and K^+ channels. We observed

decreased activity of the exchanger in the present studies, and reductions in cell membrane voltage, which will decrease NH_4^+ electrochemical driving force for its permeation through K^+ channels, have been found in multidrug-resistant cells [12,23].

The lower pH_i in LZ-8 compared to V79 cells confirms that intracellular alkalinization is not necessary for multidrug resistance [16,24,25]. Although it is possible that V79 cells, which have low levels of Pgp expression [15,16], have an increased pH_i with respect to cells without Pgp, verapamil had no effect on their pH_i, whereas it elevated LZ-8 cells pH_i. Additional support for the lack of cause-effect relationship between cell alkalinization and Pgp expression comes from other studies. Although pH_i was found elevated in a number of cells expressing Pgp [4-12], there are also reports of absence of pH_i differences between drug-sensitive and multidrug-resistant cells [16,24,25].

The response of multidrug-resistant cells to acid loads has been studied before [5,11,24], but intracellular buffering power was not measured in any of those studies, leaving open the possibility that the observed increases in the rates of pH_i recovery [5,11] were due to alterations in β_T, and not to the activity of pH_i regulatory mechanisms. Our findings of similar β_T in V79 and LZ-8 cells, and reduced rates of pH_i recovery in the latter prove that LZ-8 cells have reduced ability to extrude H^+ equivalents. Although we did not study the specific pH_i regulatory mechanisms in detail, the absence of pH_i regulation upon acid load in Hepes-buffered solutions containing 1 mM amiloride (see **Figure 5A**) suggests that Na^+/H^+ exchange is a major pH_i regulatory mechanism in V79 and LZ-8 cells. Therefore, a decrease in the Na^+/H^+ exchanger activity in the multidrug-resistant LZ-8 cells seems very likely. Since increased activity of Na^+/H^+ exchange has been reported in multidrug-resistant cells [5] and we found the opposite in LZ-8 cells, alterations in pH_i regulation may occur in different ways in different multidrug-resistant cells.

Exposure to verapamil for 1 h or less reversed the pH_i alkalinization observed in some but not all cell lines [5,6]. Our results show absence of reversing effect by 1-h exposure to verapamil on pH_i and its recovery from an acid load, but reversal upon 24-h exposure. If cells have endogenous Pgp substrates that modulate membrane transporters, the time-dependent effects of verapamil could result from differences in the relative rates of production and efflux of those substrates. The poly-specificity of Pgp and the identification of endogenous substrates [3,14] support this idea. Many alterations have been found in Pgp-expressing multidrug-resistant cells in addition to increased drug efflux and pH_i changes. These include alterations in ion transport, intracellular Ca^{2+} regulation, lipid composition, membrane trafficking, and cell-membrane voltage [11,12,23,26-33]. The mechanism of these varied alterations can be explained in part by changes in the levels of endogenous Pgp substrates, which may have different, and even opposite effects depending on the cell type. It remains to be explored whether the secondary alterations in Pgp-expressing cells modify multidrug resistance (*e.g.*, effect of pH_i on Pgp function).

Acknowledgement

We want to thank Dessa Glass for technical help. This work was supported in part by the Cancer Prevention & Research Institute of Texas Individual Investigator Award RP101073.

Abbreviations: BCECF, 2'7'-biscarboxyethly-5(6) carboxyfluorescein; β_{Bic}, HCO_3^-/CO_2 buffering power; β_i, intrinsic buffering power; β_T, total buffering power; Pgp, P-glycoprotein; pH_i, intracellular pH;

Address correspondence to: Dr. Guillermo Altenberg, Department of Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430-6551, USA; Tel: 806-743-2531; FAX: 806-743-1512; E-mail: g.altenberg@ttuhsc.edu

References

[1] Goda K, Bacsó Z, Szabó G. Multidrug resistance through the spectacle of P-glycoprotein. Curr Cancer Drug Targets 2009; 9:281-97.

[2] Seeger MA, van Veen HW. Molecular basis of multidrug transport by ABC transporters. Biochim Biophys Acta 2009; 1794:725-37.

[3] Gutmann DA, Ward A, Urbatsch IL, Chang G, van Veen HW. Understanding polyspecificity of multidrug ABC transporters: closing in on the gaps in ABCB1. Trends Biochem Sci 2010; 35:36-42.

[4] Keizer HG, Joenje H. Increased cytosolic pH in multidrug-resistant human lung tumor cells: effect of verapamil. J Natl Cancer Inst 1989; 81:706-9.

[5] Boscoboinik D, Gupta RS, Epand RM. Investi-

gation of the relationship between altered intracellular pH and multidrug resistance in mammalian cells. Br J Cancer 1990; 61:568-72.
[6] Thiebaut F, Currier SJ, Whitaker J. Haugland RP, Gottesman MM, Pastan I, Willingham MC. Activity of the multidrug transporter results in alkalinization of the cytosol: measurement of cytosolic pH by microinjection of a pH-sensitive dye. J Histochem Cytochem 1990; 38: 685-90.
[7] Roepe PD. Analysis of steady-state and initial rate of doxorubicin efflux from a series of multidrug-resistant cells expressing different levels of P-glycoprotein. Biochemistry 1992; 31: 12555-125.
[8] Luz JG, Wei LY, Basu S, Roepe PD. Transfection of mu MDR 1 inhibits Na(+)-independent Cl^-/HCO_3^- exchange in Chinese hamster ovary cells. Biochemistry 1994; 33:7239- 49.
[9] Simon S, Roy D, Schindler M. Intracellular pH and the control of multidrug resistance. Proc Natl Acad Sci USA 1994; 91:1128-32.
[10] Simon SM, Schindler M. Cell biological mechanisms of multidrug resistance in tumors. Proc Natl Acad Sci USA 1994; 91:3497-3504.
[11] Wei LY, Hoffman MM, Roepe PD. Altered pHi regulation in 3T3/CFTR clones and their chemotherapeutic drug-selected derivatives. Am J Physiol 1997; 272:C1642-53.
[12] Roepe PD. What is the precise role of human MDR 1 protein in chemotherapeutic drug resistance? Curr Pharm Des 2000; 6:241-60.
[13] Pearce HL, Winter MA, Beck WT. Structural characteristics of compounds that modulate P -glycoprotein-associated multidrug resistance. Adv Enzyme Regul 1990; 30:357-73.
[14] Wang RB, Kuo CL, Lien LL, Lien EJ. Structure-activity relationship: analyses of P-glycoprotein substrates and inhibitors. J Clin Pharm Ther 2003; 28:203-28.
[15] Sognier MA, Zhang Y, Eberle RL, Belli JA. Characterization of adriamycin-resistant and radiation-sensitive Chinese hamster cell lines. Biochem Pharmacol 1992; 44:1859-68.
[16] Altenberg, G.A., Young, G., Horton, J.K., Glass, D., Belli, J.A., and Reuss, L. Changes in intra- or extracellular pH do not mediate P-glycoprotein-dependent multidrug resistance. Proc Natl Acad Sci USA 1993; 90:9735-38.
[17] Howell N, Belli TA, Zaczkiewicz LT, Belli JA. High-level, unstable adriamycin resistance in a Chinese hamster mutant cell line with double minute chromosomes. Cancer Res 1984; 44:4023-9.
[18] Altenberg GA, Vanoye CG, Han ES, Deitmer JW, Reuss L. Relationships between rhodamine 123 transport, cell volume, and ion-channel function of P-glycoprotein. J Biol Chem 1994; 269:7145-9.
[19] Thomas JA, Buchsbaum RN, Zimniak A, Racker E. Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. Biochemistry 1979; 81:2210-8.
[20] Roos A, Boron WF. Intracellular pH. Physiol Rev 1981; 61:296-434.
[21] Ford JM. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. Eur J Cancer 1996; 32A:991-1001.
[22] Horton JK, Thimmaiah KN, Altenberg GA, Castro AF, Germain GS, Gowda GK, Houghton PJ. Characterization of a novel bisacridone and comparison with PSC 833 as a potent and poorly reversible modulator of P-glycoprotein. Mol Pharmacol 1997; 52:948-57.
[23] Vayuvegula B, Slater L, Meador J, Gupta S. Correction of altered plasma membrane potentials. A possible mechanism of cyclosporin A and verapamil reversal of pleiotropic drug resistance in neoplasia. Cancer Chemother Pharmacol 1988; 22:163 8.
[24] Litman T, Pedersen SF, Kramhøft B, Skovsgaard T, Hoffmann EK. pH regulation in sensitive and multidrug resistant Ehrlich ascites tumor cells. Cell Physiol Biochem 1998; 8:138 -50.
[25] Frezard F, Garnier-Suillerot A. Determination of the osmotic active drug concentration in the cytoplasm of anthracycline-resistant and -sensitive K562 cells. Biochim Biophys Acta 1991; 1091:29-35.
[26] Sehested M, Skovsgaard T, van Deurs B, Winther-Nielsen H. Increased plasma membrane traffic in daunorubicin resistant P388 leukaemic cells. Effect of daunorubicin and verapamil. Br J Cancer 1987; 56:747-51.
[27] Yamashita N, Hamada H, Tsuruo T, Ogata E.Enhancement of voltage gated Na^+ channel current associated with multidrug resistance in human leukemia cells. Cancer Res 1987;47:3736-41.
[28] Fritz F, Howard EM, Hoffman MM, Roepe PD. Evidence for altered ion transport in Saccharomyces cerevisiae overexpressing human MDR 1 protein. Biochemistry 1999; 38:4214-26.
[29] Vanoye CG, Castro AF, Pourcher T, Reuss L, Altenberg GA. Phosphorylation of P-glycoprotein by PKA and PKC modulates swelling-activated Cl^- currents. Am J Physiol 1999; 276:C370-8.
[30] Porcelli AM, Scotlandi K, Strammiello R, Gislimberti G, Baldini N, Rugolo M. Intracellular pH regulation in U-2 OS human osteosarcoma cells transfected with P-glycoprotein. Biochim Biophys Acta 2002; 1542:125-38.
[31] Mizutani T, Masuda M, Nakai E, Furumiya K, Togawa H, Nakamura Y, Kawai Y, Nakahira K, Shinkai S, Takahashi K. Genuine functions of P-glycoprotein (ABCB1). Curr Drug Metab 2008; 9:167-74.
[32] Sulová Z, Seres M, Barancík M, Gibalová L, Uhrík B, Poleková L, Breier A. Does any rela-

tionship exist between P-glycoprotein-mediated multidrug resistance and intracellular calcium homeostasis? Gen Physiol Biophys 2009; 28:F89-95.

[33] Poulsen KA, Andersen EC, Hansen CF, Klausen TK, Hougaard C, Lambert IH, Hoffmann EK. Deregulation of apoptotic volume decrease and ionic movements in multidrug-resistant tumor cells: role of chloride channels. Am J Physiol Cell Physiol 2010; 298:C14-25.