SHORT COMMUNICATION

Plasmalemmal pH-Gradients in Drug-Sensitive and Drug-Resistant MCF-7 Human Breast Carcinoma Xenografts Measured by 31P Magnetic Resonance Spectroscopy

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ABSTRACT. 31P Magnetic resonance spectroscopy (MRS) was employed to investigate tumor pH in xenografts of drug-sensitive and drug-resistant MCF-7 human breast carcinoma cells. Measured extracellular pH values were found to be lower than the intracellular pH in all three tumor types investigated. The magnitude of this acid-outside plasmalemmal pH gradient increased with increasing tumor size in tumors of two drug-resistant variants of MCF-7 cells, but not in tumors of the parent (drug-sensitive) cells. The partitioning of weak-base or weak-acid drug molecules across the plasma membrane of a tumor cell is dependent upon the acid-dissociation constant (pK\textsubscript{a}) of the drug as well as the plasmalemmal pH gradient. A large acid-outside pH gradient can exert a protective effect upon the cell from weak-base drugs such as anthracyclines and Vinca alkaloids, which have pK\textsubscript{a} values of 7.5 to 9.5. The possibility of enhancing the therapeutic efficacy of weak-base drugs by dietary or metabolic manipulation of the extracellular pH, in order to reduce or reverse the plasmalemmal pH gradient, deserves investigation. BIOCHEM PHARMACOL 57;3:309 –312, 1999. © 1998 Elsevier Science Inc.

KEY WORDS. MCF-7; breast carcinoma; xenograft; tumor pH; 31P magnetic resonance spectroscopy; 3-aminopropylphosphonate

Combination chemotherapy of breast cancer and other cancers usually involves the use of at least one partially ionizable drug species [1]. The partitioning of weak-base or weak-acid drug molecules across the plasma membrane of a tumor cell is dependent upon the pK\textsubscript{a} of the drug as well as the plasmalemmal pH gradient. A large acid-outside pH gradient can exert a protective effect upon the cell from weak-base drugs such as anthracyclines and Vinca alkaloids, which have pK\textsubscript{a} values of 7.5 to 9.5 [2–5]. The pH of tissues, including tumors, has been measured most commonly by the use of microelectrodes [6]. The pH thus measured is generally a combined measurement of the pH of interstitial fluid, and fluid and blood from damaged cells and capillaries. The invasive nature of microelectrodes and the uncertainty as to the nature of the interrogated fluid space complicate their use. In contrast, 31P MRS can be employed to non-invasively, and simultaneously, measure pH\textsubscript{i} from the chemical shift of endogenous inorganic phosphate [7] and pH\textsubscript{e} from the chemical shift of exogenous 3-APP [8]. We have employed 31P MRS to investigate differences in the steady-state plasmalemmal pH gradients in xenografts of drug-sensitive and drug-resistant MCF-7 human breast carcinoma cells. The pH\textsubscript{i} and pH\textsubscript{e} of all cell lines decreased with increasing tumor size. However, the (pH\textsubscript{i}−pH\textsubscript{e}) gradient was observed to increase with increasing tumor size only in tumors of two drug-resistant variants of MCF-7 cells, and not in tumors of the parent (drug-sensitive) cells. Implications of an acid-outside pH gradient for chemotherapy with weak-base drugs are discussed.

MATERIALS AND METHODS

Cells and Animals Used

MCF-7/S cells were obtained from the Michigan Cancer Foundation. MCF-7 cells resistant to mitoxantrone (MCF-7/Mitox) and doxorubicin (MCF-7/D40) were generated by sequential culturing in increased concentrations of mitoxantrone and doxorubicin, respectively [9]. SCID mice were obtained from the University of Arizona SCID mouse resource. Cells were implanted in the mammary fat pads of...
6- to 7-week-old female SCID mice as a suspension of 5 × 10⁶ cells in 0.05 mL of Hanks’ balanced salt solution containing 50% Matrigel (Collaborative Research). As these cells are estrogen-dependent, 17β-estradiol pellets (0.25 mg, 21-day release; or 0.75 mg, 60-day release; Innovative Research of America) were implanted subcutaneously in the shoulder region of the mice by means of a 12-gauge trocar (Innovative Research) 2 days prior to tumor inoculation. In the case of the 21-day release pellets, a new pellet was implanted every 3 weeks, as necessary.

In vivo MRS

Tumors were allowed to grow for 3–8 weeks to volumes of 150–1500 mm³, as estimated by external measurements of the tumor. Tumor volumes were calculated from orthogonal measurements of the external dimensions of tumors using the formula (width)² × length/2 [10]. Prior to MRS, the mice were anesthetized with a combination of ketamine (72 mg/kg), xylazine (6 mg/kg), and acepromazine (6 mg/kg). A 3/4 in., 24-gauge catheter (Elf Sanofi Inc.) connected to a 1.58 mm i.d. polyethylene tube (Becton Dickinson) long enough to extend out of the magnet was inserted into the intraperitoneal cavity of the anesthetized animal. 3-APP was obtained from the Sigma Chemical Co. A solution of 3-APP (0.15 to 0.3 mL, 128 mg/mL, pH 7.4) could be remotely injected into the mouse at the appropriate time before spectroscopy via the i.p. catheter. All in vivo measurements were performed at 4.7 Tesla (T) on a Bruker Biospec spectrometer/imager. The mouse was immobilized on a home-built probe, with the whole tumor placed inside a 3-turn solenoid radiofrequency (rf) coil of appropriate diameter—8, 12, or 17 mm—tunable to ¹H or ³¹P. Body temperature was maintained by a circulating water blanket placed under the immobilized mouse. Unlocalized ³¹P MR spectra were acquired using 20–45° pulses with repetition times of 500–1000 msec. Volume-selective ³¹P spectra were acquired using either VSEL, a double-refocused spin-echo implementation of the VOSY pulse sequence [11] provided by Bruker Medizintechnik, or the ISIS sequence [12]. Scout images of the tumors were acquired prior to localized spectroscopy, to guide voxel placement. VSEL spectra were acquired using 764 μsec slice-selective hermitian rf pulses (corresponding to 80 ppm in the ³¹P MR spectrum), an echo time of 11 msec, and a repetition time of 1200 msec. ISIS spectra were acquired with adiabatic slice-selective and excitation pulses repeated every 10–12 sec, using a gradient strength of 75 mT/m. In all cases, a dwell time of 62.5 μsec was employed, and 8192 data points were collected. Transients were averaged for 10–30 min. The large spectral widths employed resulted in up to a 1-mm difference in the positioning of the voxel containing either α-NTP or 3-APP, and the voxel containing the central frequency. This chemical shift artifact was not corrected for, but voxel sizes and placement were chosen so as to minimize the contribution of signal arising from the mouse body wall, while covering as much of the tumor as possible.

pH Calibration of Spectral Peaks

³¹P MR time-domain data were processed by exponential multiplication followed by Fourier transformation. Chemical shifts of 3-APP and inorganic phosphate were calibrated to the α peak of NTP (set to −10.05 ppm). Titration curves reported elsewhere for the pH-dependencies of the chemical shifts of 3-APP [13, *] and inorganic phosphate [14] were used to calculate pHₑ and pHᵢ, respectively, from each ³¹P MR spectrum. The intensity of each 3-APP peak was corrected for the non-linearity of the titration curve prior to assigning a pH value to the chemical shift of the top of the peak [15]. Figure 1 shows a representative ³¹P MR spectrum of an MCF-7/S tumor obtained using the ISIS sequence.

Statistical Analyses

Tumor pHₑ and pHᵢ were obtained from ³¹P MR spectra of several MCF-7/S, MCF-7/D40, and MCF-7/Mitox tumors. A linear regression analysis was performed for data sets from each tumor type, in order to determine the nature of the relationship between tumor pHₑ (or pHᵢ) and tumor volume. The values and 95% confidence intervals for the first-order regression parameters were calculated using Sigmastat (Jandel Corp.).

RESULTS AND DISCUSSION

³¹P MR Spectrum

Figure 1 shows a representative ³¹P MR spectrum of a 610 mm³ MCF-7/S tumor, obtained from an 8 × 8 × 8 mm³ voxel within the tumor, pHₑ and pHᵢ were calculated from the chemical shifts of the 3-APP and inorganic phosphate peaks, respectively, as described in Materials and Methods.

Variation in pHₑ and pHᵢ with Tumor Size

Panels a–c of Fig. 2 display the tumor pHₑ and pHᵢ measured from localized and unlocalized ³¹P MR spectra of three different tumor types, as a function of tumor volume. Also displayed are the results of first-order linear regression curve fits for each data set. Table 1 shows the values and the 95% confidence intervals for the fitted slopes, obtained from a linear regression analysis of each data set. The data clearly show that tumor pHₑ and pHᵢ decrease with increasing tumor size.

Variation of Plasmalemmal (pHₑ–pHᵢ) Gradient with Tumor Volume

The first-order fitted lines shown in Fig. 2a indicate that for the drug-sensitive MCF-7/S tumors, the magnitude of the

(pHᵢ–pHe) gradient changed little with increasing tumor volumes. The pHᵢ dropped concomitantly with the drop in pHe. The overlapping 95% confidence intervals for the slopes of the pHe and pHᵢ data sets for the MCF-7/S tumors (Table 1) verify that the (pHᵢ–pHe) gradient was not altered significantly. From panels b and c of Fig. 2, it can be seen that the fitted lines are divergent for the pHᵢ and pHe values of MCF-7/D40 and MCF-7/Mitox tumors. Thus, for these tumors, the magnitude of the (pHᵢ–pHe) gradient increased as the tumors grew. The 95% confidence intervals for the fitted slopes for the pHe and pHᵢ data sets from both MCF-7/D40 and MCF-7/Mitox tumors did not overlap.

FIG. 1. ³¹P MR ISIS spectrum acquired from an 8 x 8 x 8 mm³ voxel placed on a 610 mm³ MCF-7/S tumor. Adiabatic excitation pulses were used to minimize artifacts arising from non-uniform spatial excitation; 8192 data points were collected over a spectral window of 8000 Hz, from a total of 184 averages with a recycle delay of 10.5 sec. An exponential line-broadening factor of 5 Hz was applied to the time-domain signal prior to Fourier transformation. Pᵢ, inorganic phosphate; NTP, nucleoside triphosphate; and PME, phosphomonooesters. pHᵢ = 7.11, pHᵢ = 7.17.

FIG. 2. Variation in pHᵢ and pHe with tumor size. (a) MCF-7/S tumors, (b) MCF-7/D40 tumors, and (c) MCF-7/Mitox tumors. Key: (●) pHᵢ data, (○) pHe data, (—) pHᵢ linear regression, and (···) pHe linear regression.
(Table 1), indicating that the slopes of the pH$_e$ and pH$_i$ regressions for these two tumor types were significantly different. Furthermore, the pH$_e$ slope for MCF-7/Mitox tumors was significantly steeper than that for MCF-7/D40 tumors.

It is thus clear that (i) a positive (pH$_i$–pHe) gradient exists in tumors of all three variants of MCF-7 cells, and (ii) the magnitude of this gradient increases with increasing tumor volume in the two drug-resistant variants, but not the drug-sensitive parent tumor type. A pH gradient in this direction may confer a measure of protection to the tumors from weak-base drugs like doxorubicin and mitoxantrone, by partial exclusion of the drugs from the cytosol [2–5]. This would be a form of “physiological” drug resistance, as distinct from the better characterized “biochemical” drug resistance observed, for example, in cells expressing the P-glycoprotein drug pump (reviewed in Ref. 16). The increased magnitude of the plasmalemmal pH gradient in MCF-7/D40 and MCF-7/Mitox tumors at the larger tumor sizes may partially explain why the large tumors are refractory to drug therapy (unpublished results). While such a pH gradient would seem to favor the use of weak-acid drugs such as chlorambucil, these are not commonly used. The possibility of enhancing the therapeutic efficacy of commonly used weak-base drugs by dietary or metabolic manipulation of pH$_e$ in order to reduce or reverse the plasmalemmal pH gradient deserves investigation.

References


TABLE 1. Slopes and 95% confidence intervals for the tumor pH versus the tumor volume data sets shown in Fig. 2

<table>
<thead>
<tr>
<th>Data set</th>
<th>Fitted slope (10$^{-4}$ pH units/mm$^3$)</th>
<th>95% Confidence interval (10$^{-4}$ pH units/mm$^3$)</th>
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<tbody>
<tr>
<td>MCF-7/S, pH$_e$</td>
<td>−3.4</td>
<td>−1.9 to 4.9</td>
</tr>
<tr>
<td>MCF-7/S, pH$_i$</td>
<td>−3.0</td>
<td>−1.8 to 4.2</td>
</tr>
<tr>
<td>MCF-7/D40, pH$_e$</td>
<td>−4.3</td>
<td>−3.5 to 5.0</td>
</tr>
<tr>
<td>MCF-7/D40, pH$_i$</td>
<td>−1.5</td>
<td>−0.98 to 2.1</td>
</tr>
<tr>
<td>MCF-7/Mitox, pH$_e$</td>
<td>−6.6</td>
<td>−5.6 to 7.5</td>
</tr>
<tr>
<td>MCF-7/Mitox, pH$_i$</td>
<td>−1.6</td>
<td>−0.93 to 2.2</td>
</tr>
</tbody>
</table>

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