**Advances in Brief**

**Rapid in Vivo Monitoring of Chemotherapeutic Response Using Weighted Sodium Magnetic Resonance Imaging**

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**Abstract**

A novel pulse sequence strategy uses sodium magnetic resonance imaging to monitor the response to chemotherapy of mouse xenograft tumors propagated from human prostate cancer cell lines. An inversion pulse suppresses sodium with long longitudinal relaxation times, weighting the image toward intracellular sodium nuclei. Comparing these weighted sodium images before and 24 h after administration of antineoplastics, we measured a 36 ± 4% \( (P < 0.001; n = 16) \) increase in signal intensity. Experiments with these same drugs and cells, treated in culture, detected a significant intracellular sodium elevation \( (10–20 \text{ mM}) \) using a ratiometric fluorescent dye. Flow cytometry studies showed that this elevation preceded cell death by apoptosis, as determined by fluorescent end-labeling of apoptotic nuclei or Annexin V binding. Histopathology on formalin-fixed sections of explanted tumors confirmed that drug administration reduces proliferation \( (2.2 \text{ versus } 8.6 \text{ mitotic figures per high power field}; P < 0.0001) \), an effect that inversely correlates with the sodium magnetic resonance image response on a tumor-to-tumor basis \( (P < 0.02; n = 10) \). Morphological features, such as central zones of nonviable cells, rims of active apoptosis, and areas of viable tumor, could be distinguished by comparing weighted and unweighted images. Advantages of this sodium imaging technique include rapid determination of drug efficacy, improved diagnosis of lesions, ease of coregistration with high resolution proton magnetic resonance imaging, and absence of costly or toxic reagents.

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3 The abbreviations used are: MRI, magnetic resonance imaging; SQ, single quantum; MQ, multiple quantum; EC, extracellular; IC, intracellular; IR, inversion recovery; VP-16, etoposide; HPF, high power field.

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**Introduction**

*In vitro* cytotoxicity assays provide preliminary information about the activity of an antineoplastic agent against a particular solid tumor, but these techniques are limited by the difficulties in obtaining and culturing human cells in explant. It may take weeks to determine the success or failure of a specific chemotherapeutic activity based on change in size of a soft tissue lesion. Therefore, some patients have ineffective drugs administered needlessly until clear progression is observed in a computed tomography scan or proton MRI or clinical symptoms worsen. Apoptosis and related cellular changes, however, can be observed in *vitro* within hours of exposure to antineoplastic agents. An *in vivo* assay of chemotherapeutic efficacy based on such rapid changes would significantly contribute to patient management by providing real-time drug efficacy information so that therapy could be optimized and ineffective therapy discontinued. Hence, various recent studies have focused on this problem using changes in F-18 fluorodeoxyglucose uptake [as measured with positron emission tomography imaging (1)] and changes in cell metabolism [as measured with P-31 MR spectroscopy (2–4)] in an effort to obtain noninvasive, real-time information on therapeutic effect. However, being able to accomplish this goal using higher resolution magnetic resonance imaging would have enormous clinical benefit. The novel application of MRI that we describe uses sodium nuclei and weights sodium MR images toward populations of sodium nuclei that are physiologically relevant to detecting tumors and monitoring their treatment. In addition to detecting rapid cellular events, this sodium MRI approach has the additional advantage of ready coregistration with high resolution proton MRI, allowing for comparison with tissue structure, angiography, and imageable gene markers.

Because of the biological importance of sodium, its relative abundance, and its sensitivity, sodium NMR is a particularly useful tool for the study of pathophysiological processes. The large transmembrane ionic gradient causes the much lower IC concentration \([\text{Na}^+]_\text{i}\) to be highly dependent on active processes \((\text{Na}^+/\text{K}^+\text{-ATPase pump})\) and thus responsive to metabolic suppression. With special relevance to the study of neoplasms, \([\text{Na}^+]_\text{i}\) is correlated with the proliferation rate of nonneoplastic and malignant cell populations (5), presumably because of the role of \(\text{Na}^+\) influx in initiating movement through the cell cycle. \(\text{Na}^+\) flux is mediated by transmembrane exchangers for both \(\text{Ca}^{2+}\) and \(\text{H}^+\) (6, 7). Thus, not only is there probable elevation of \([\text{Na}^+]_\text{i}\), because of the toxic effects of chemotherapy, but the
altered values of baseline [Na], if detectable, could also have diagnostic significance.

Measurement of sodium content clinically has typically been done using SQ NMR techniques (8, 9). A significant disadvantage of SQ NMR is the relatively larger abundance of EC versus IC [Na]. SQ NMR requires paramagnetic shift reagents to discern Na. Because of their relative membrane impermeability, they selectively shift the MR spectrum of the EC sodium. However, shift reagents would have severe disadvantages, including toxicity, in clinical use. An alternative MR approach to measure IC sodium content is based on the interaction of sodium polyions and their resultant effects on nuclear spin transitions. Spin 3/2 nuclei (such as Na and K) have a nonvanishing quadrupole moment, allowing interaction with electrostatic field gradients (10). In certain complex environments, such as those occurring in IC space, MQ spin transitions occur that can be detected by specific pulse sequences called MQ filters (11–14). The EC sodium diffuses relatively freely, with a small but presumed constant component electrostatically bound to plasma proteins and the surface of cell membranes. Thus, the presence of an MQ signal can be used to identify populations of sodium nuclei by their molecular environment and can be used to detect [Na], changes (15–22).

In this study, we capture this utility of MQ sodium MRI, the ability to identify sodium by its microenvironment, but use instead a SQ pulse sequence. The higher signal-to-noise of SQ sodium MRI is a distinct clinical advantage. The sodium subpopulation weighting is accomplished by applying an IR pulse sequence to null the signal from sodium nuclei with long longitudinal relaxation times (T1), as found in free solution or plasma. The image signal is then weighted toward bound sodium, which is primarily IC. The IR technique is commonly used in proton imaging to distinguish fat and water. This study was designed to test the adequacy of such a weighting scheme and whether images so derived would have the hypothesized diagnostic utility.

Materials and Methods

Cell Culture and Tumor Propagation. PC3 and DU145 human prostate cancer cell lines were obtained from American Type Culture Collection (Rockville, MD) and cultured in RPMI 1640 with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 1 mM nonessential amino acid. To propagate PC3 and DU145 cell lines as solid tumors, we mixed 1 ml of ice-cold Matrigel (Biomedical Products Division, Bedford, MA; Matrigel is an in vivo substrate that is liquid at 4°C and solid at 37°C), with 4 × 10^6 cells suspended in 1 ml of ice-cold media. We then injected each homozygous Taconi NCR male nude mouse with 0.5 ml of the mixture s.c.

Flow Cytometry and Fluorescent Markers for Apoptosis. Fluorescent end-labeling techniques for flow cytometry were as described (23). Briefly, cells were fixed in ice-cold methanol free formaldehyde (1% in PBS), resuspended in 70% ethanol, and stored at −20°C (24). Kit directions were followed (APO-BrdU; Phoenix Flow Systems, San Diego, CA). For Annexin labeling, cells were treated, pelleted, and exposed to Annexin V/FITC conjugated antibody. (Chemicon International; Ref. 25). Trypsin, which cleaves the antibody binding site, was avoided. Flow cytometry was performed on a Becton/Dickinson FACStar II (APO-BrdU) or exCaliber flow system (Annexin) at the Columbia University Cancer Center and analyzed using Winmdi Software.

Ionic Activity Measurements with Ratiometric Dyes. For fluorescent ion measurements (free [Na] and [Ca]), cells were plated in 96-well, low autofluorescence culture plates (Costar). After treatments, wells were washed twice with PBS and covered for 1 h with 125 μl of dye loading buffer: (a) for [Ca], measurements, 50 μg Fura II/acetoxymethylster (26) was added with 30 μl pluronic to 6 ml of clear DMEM; (b) for [Na], measurements, Fura II/AM was replaced by 10 μg of SBF acetoxymylester (27, 28) and loading continued for 3 h (dyes from Molecular Probes, Eugene, OR). Wells were then washed twice with PBS (plus 1.0 mmol/l Ca^{2+}, 0.6 mmol/l Mg^{2+}, and 2 mg/ml dextrose).

For [Na], an in situ calibration technique was used whereby cells were permeabilized to Na+ with monensin (20 μM) and gramicidin (40 μM); [Na], was varied in EC solutions while adjusting [K]_m, [Cl]_m, and isothionate as described (27, 28). For [Ca], measurements, one row of wells was exposed to ionomycin (10 μM) and one to ionomycin plus EGTA (20 mM) to derive R_{max} and R_{min} (K_d was assumed to be 371 nM). The average control concentration for the five experiments was 126 nm. We displayed the percentage of change in control concentration calculated from the ratio measurements (26). For ion measurements, plates were run on a Fluoroskan II fluorescent plate reader (Titertek). Ratios were derived by dividing fluorescent intensity at 345 nm excitation by that at 390 nm, both with a 508-nm emission filter. For all plates, dye-free wells were examined for both control and drug-treated cells and served as background values. For [Ca], experiments, exposure to 6 mM MnCl_2, which quenches dye, was an added control (26). Averaged background values were subtracted from each well in the protocol grouping prior to deriving the ratio. Thapsigargin was added as a positive control for [Ca], elevation and generated rapid elevations in excess of 1 μM.

MRI and Analysis. All imaging experiments were performed on a high field (4.23 Tesla) whole body MRI system at the Columbia University Hatch NMR Center. A small quadrature birdcage radiofrequency coil (50-mm inside diameter; Morris, Inc.) and a high-strength gradient insert coil (30 mT/m; Bruker model G-33) were used in this study. Acquisition parameters were: TR, 100 ms; TE, 5.6 ms; FOV, 40 mm; slice thickness, 2.5 mm; inversion time, 25 ms; and flip angle, 90°. Acquisition matrix was 64 × 64 × 8. Both inversion and excitation pulses were nonselective.

Two reference phantoms of 200 mmol/l NaCl with either 30% (P1) or 40% Ficoll (P2) were glued to a plastic animal platform. The anesthetized mouse was aligned in a deep groove with the tumor positioned through a small elliptical opening in the plastic holder, thus maintaining the same relative position to the phantoms from experiment to experiment. The 40% phantom is the brightest phantom on the IR image, and the 30% phantom is the brightest on the SQ image. IR images and SQ images were normalized to the brightest phantom within each slice.

Analysis of tumor intensity change was measured at the brightest region using line profiles or region-of-interest res-
ident software. This was generally at the center for uniformly bright images or on the largest portion of the annulus. To confirm tumor and kidney positions, paired proton and sodium quadrature birdcage coils, identical in dimension, were used.

Administration of Chemotherapy. Drug was dissolved in 100–150 ml of vehicle and slowly injected into a femoral vein under microscopic observation and while the mouse was anesthetized with ketamine and xylazine. We injected 10 mg/ml Taxotere (i.e., 1.0 mg) or 2 mg/ml VP-16 (0.3 mg). The reported toxic mouse dose for Taxotere is 4.5 mg. The mouse doses equivalent to human doses were calculated from standard equations that convert weight to surface area for small mammals. Because of problems with viscosity for the mouse i.v. injections, the VP-16 dose was about one-third the calculated equivalent human dose. All animals were used in accordance with Institutional Animal Care and Use Committee and Columbia University rules for humane treatment of animals.

Tumor Postmortem. Formalin-fixed, paraffin-embedded sections of the explanted neoplasms were cut at 4 µm and stained with H&E or deparaffined and fluorescently end-labeled (100-µl aliquots of APO-BrdU kit reagents applied directly to slides in a moist environment). The number of mitotic figures was enumerated in up to 20 HPFs (×400) in the rim of viable cancer cells for each specimen. Care was taken not to include central areas with cellular debris or foci with back-to-back apoptotic cells. In some specimens, <20 HPFs were available for evaluation. The mitotic index represents the average number of mitotic figures per HPF (29, 30)

Results

Sodium Inversion Recovery Can Null Signal from Selected T1 Range. To produce the weighted sodium image, an IR pulse sequence is used to suppress the signal from sodium nuclei with long $T_1$ relaxation times (corresponding to free sodium). This technique selects populations of sodium nuclei based on longitudinal relaxation time ($T_1$), as opposed to the transverse relaxation time ($T_2$) used in the standard MQ approach. In general, to suppress the contribution of sodium nuclei with a range of $T_1$ values about some $T_{1ex}$, one sets the inversion time (the time between the 180° and 90° pulses in the IR pulse sequence) to $(\ln 2)(T_{1ex})$. This is a good approximation for long repetition times. Because of the higher concentration of total sodium and lower proportion of bound sodium in the EC space, an IC weighted sodium signal and image are produced.

The ability to weight for different populations of sodium nuclei is easily demonstrated using a cell-free system, e.g., phantoms. The phantoms were tubes of NaCl solution. Some contained Agar or Ficoll to simulate the increased viscosity and electrostatic binding of sodium, which occurs to some extent in the EC space but more extensively in the IC space. With appropriate choice of inversion time, depending on the concentration of Agar or Ficoll, the signal from a particular test phantom can be totally suppressed. We imaged two phantoms (with and without 4% Agar) at nine different inversion times, and in the absence of an inversion pulse, and plotted the intensity for each image as a separate point. Complete suppression was obtained at inversion times of 19 and 31 ms, respectively (Fig. 1). The straight-line is a fit of the theoretical relationship

![Fig. 1](https://example.com/)
between image intensity, $T_1$ and inversion time (assuming $T_1$ values of 27 and 43 ms). This exercise demonstrates how one can totally suppress the signal from a particular homogeneous population of sodium nuclei based on relaxation times.

**Application of Sodium IR to Mouse Tumors.** To determine the best pulse sequence for suppressing EC sodium, different inversion times were tested on a human tumor xenograft mouse model of prostate cancer. We chose 25 ms as the optimum inversion time for several reasons. For this inversion time, the signal was totally nulled for a cystic tumor (e.g., a tumor sac that was predominantly fluid; Fig. 1, insets). Furthermore, the mouse kidneys, which appeared very bright on the SQ MR image with no inversion pulse, were also totally suppressed on the IR image (Fig. 2, compare C and D). This inversion pulse, therefore, was quite effective at nulling EC bulk fluid.

This same pulse sequence (using a 25-ms inversion pulse), which completely suppressed EC fluid signal, was also ideal for enhancing the visibility of solid tumors, which appeared relatively brighter in comparison with the mouse body. This is presumably because of the higher IC sodium in tumor versus normal tissue (see “Discussion”) and the ability of this pulse sequence to enhance the contribution of IC sodium. Compare the IR sodium images (Fig. 2, B and D) with traditional (no inversion pulse) SQ sodium images (Fig. 2, A and C) for solid tumors formed from two human cell lines (DU145, Fig. 2, A and B; PC3, Fig. 2, C and D). Thus, 25 ms is the optimal inversion time for both suppressing EC sodium nuclei as well as relatively enhancing the image of solid tumors at the magnetic field strength (4.23 Tesla) used in our experiments. Note that the tumor is also bright on the traditional SQ image (see “Discussion”).

**Intracellular Ion Elevation during Drug-induced Apoptosis in Culture.** The dissipation of the transmembrane sodium gradient during cell necrosis has been well studied. Less well studied are changes in ionic gradients during apoptosis. To examine the relationship between sodium elevation and the extent of apoptotic cell progression in a controlled setting, cultured PC3 (and in some cases DU 145) cells were exposed to the two drugs used in the in vivo experiments, i.e., Taxotere (which disrupts microtubule assembly), and VP-16 (a topoisomerase inhibitor). Apoptosis was assessed with flow cytometry using both fluorescent end-labeling of DNA fragments and Annexin V binding (Fig. 3, A and B). [Na$^+$] elevation was examined using the fluorescent ratiometric dye, SBFI/AM (Fig. 3C). Because Ca$^{2+}$ is coupled to Na$^+$ by various membrane exchangers and is linked to cell death and apoptosis, free concentration ([Ca$^2+$]) was also measured using a different ratiometric dye, Fura II/AM (Fig. 3D). Because [Ca$^2+$] elevation would be

![Fig. 2](image-url) Effects of IR pulses on tumor images. A 24-slice three-dimensional gradient-echo SQ image and a 24-slice IR image were acquired in 15 and 45 min, respectively. One slice each from a DU145 tumor (A and B) and PC3 tumors (C and D) show noninverted SQ (left panels, A and C), and IR acquisitions (right panels, B and D). Tumors (straight arrows) are bright in all image sets but are dominant in the IR images. Note that kidneys (C, curved arrows) are bright in the SQ image but completely suppressed in IR image (D). The tumor is directly below the mouse body, which is delineated in red or yellow, and confirmed by coregistered proton image (not shown). The phantoms (P1 and P2; see “Materials and Methods”) have different $T_1$ values and respond differently to the inversion sequence. Acquisition parameters are described in “Materials and Methods.”
expected to accompany [Na] elevation, this additional measurement served as an independent confirmation of ionic response.

At the concentrations selected (10 nM Taxotere and 10 μg/ml VP-16), end-labeling fluorescence was not seen at 8 h and was not fully developed until 24–48 h in PC3 cells. The only rapid response for end-labeling fluorescence (within 24 h) was seen with the combination of VP-16 and DU145 cells (Fig. 3A). In PC3 cells, the earliest Annexin V response was 5 h (VP-16), but it required an additional 24 h (VP-16) or 48 h (Taxotere) to fully develop at the drug concentrations used (Fig. 3B). The ionic elevations (approximated at 10–20 mM for [Na] and 150 mM for [Ca]) were already significant as early as 2–6 h and peaked at 6–24 h (Fig. 3C and D). Thus, drug-induced ionic elevation preceded the development of apoptotic markers, suggesting that the ionic responses are not a trivial manifestation of the loss of membrane integrity associated with cell death.

Increased Intensity of IR Sodium Image during Drug Effects in Vivo. The effect of chemotherapy on the IR sodium image intensity of in vivo tumors was consistent with the culture results, i.e., IR sodium image intensity increased after in vivo drug administration. This is suggestive of increased [Na]. Control and posttherapy images were acquired in 16 mice. Tumors in two additional mice had images reacquired after control saline injections. Taken as a whole, the tumors exhibited increased intensity (36% ± 6%) after chemotherapy (P < 0.001; n = 16), a response significantly different (P < 0.002) than that after saline injections (−5% ± 4%; n = 4; not significant versus control at either 24 or 48 h). Taken separately, the change for each drug on PC3 cells was significant; Taxotere induced a 32% increase (P < 0.001; n = 5; Fig. 4, top left) and VP-16 induced a 38% change (P < 0.0005; n = 7; Fig. 4, bottom left). The responses grouped by drug were not significantly different from each other (P > 0.15). The average signal at 48 h was still elevated compared with control (28%, P < 0.05; n = 11) for the PC3 cell tumors. Four experiments using a second human prostate cancer cell line (DU145; two each with Taxotere and VP-16) also showed significant elevation at 24 h taken as a group (34%; P < 0.05; n = 4). An independent measure of drug effect, reduction in mitotic index, is plotted in the right panel and is shown to correlate with image enhancement (see below).
Independent Assay of Drug Effect Using Histological Measure of Proliferation. In an effort to determine the origins of the IR image and enhanced intensity and to verify that the drugs attained therapeutic levels, 10 of the imaged tumors were explanted after final imaging sessions and examined. On the basis of morphology, the neoplastic tissue was divided into three concentric zones, which included a central zone with cellular debris and absence of cell nuclei, surrounded by a rim of back-to-back apoptotic cells. An outermost peripheral zone was viable tumor tissue. The border between the viable and nonviable tissue could be clearly seen in HPFs, where a rim of densely packed apoptotic cells was clearly visible abutting the surviving rim of viable cells.

In HPFs of the surviving rims of these tumors (Fig. 5), one can see that the untreated tumor (Fig. 5A) has more mitotic figures than the treated tumor (Fig. 5B). Because a reduction in proliferation, attributable to cell cycle arrest, is a useful indicator of antineoplastic efficacy (29, 30), the incidence of mitotic figures was measured for up to 20 HPFs in the surviving rim of each tumor. Mitotic figures were reduced significantly ($P < 0.0001$) in the treated ($n = 7$) tumors [2.24 ± 0.163 (SE) per field; $m = 127$ fields] versus untreated ($n = 3$) tumors [8.57 ± 0.84 per field; $m = 47$]. Importantly, there was, among all treated tumors, a significant inverse correlation ($P < 0.02$) between the reduction in mitotic figures and the relative enhancement in the IR sodium image (Fig. 4, right panel).

Correlation of Image Configuration with Histology and Immunofluorescence. Because the tumor was explanted in this study after the final imaging session, which was not always at the point of peak image enhancement, we are limited in our postmortem analysis. Nevertheless, we can draw tentative conclusions about the origin of the bright entities on the IR images.

Pre- and postdrug IR images are shown along with the control SQ image for a tumor with significant central necrosis. Tumors with significant central necrosis had a characteristic dark-center/bright-annulus presentation on the inversion image (Fig. 6, A and B). These tumors were nevertheless uniformly bright on the SQ image (no inversion; Fig. 6C). Note that the effect of drug exposure (enhanced image intensity) is clearly bright on the SQ image (no inversion; Fig. 6C). Among treated tumors, there is a significant negative correlation between mitotic figures and sodium IR image intensity ($P < 0.02$), which is indicated by the regression line. (For acquisition parameters, see “Materials and Methods.”)
a clear increase in the diameter of the area of brightness on the IR image, which occurred between 24 and 48 h after treatment. We examined this region of recent IR brightening under higher power. Evidence of a well-defined rim of apoptotic cells just under the surviving rim was visible in adjacent tissue sections stained either with H&E (Fig. 7D) or immunofluorescent end-labeling (Fig. 7E). We examined the fluorescent staining; the cells just under the surviving rim were brightest, and the cells closer to the small nonviable center were less bright. Presumably, the most brightly stained cells most recently underwent apoptosis, and the less bright cells had more time to lose their fragmented DNA. Thus, cells with no nuclear staining on H&E micrographs were present only in the small center of tumor M (300 μm). In contrast, tumor Q showed a large center of cells without nuclei. On the basis of the image broadening and immunofluorescence, a plausible interpretation is that a portion of the inner surviving rim was converted to a zone of back-to-back apoptotic cells between 24 and 48 h. Thereafter, but not yet at the time of tumor removal, cellular debris would have accumulated in the center, as seen with Q.

Discussion
These results are consistent with reports that ratios of IC Na⁺ versus K⁺ are higher in both benign and malignant tumors than in their normal cellular counterparts (31, 32). In vitro tissue studies further showed that [Na], is elevated by as much as a factor of two in neoplastic compared with normal tissue, malignant compared with benign tumor, and poorly differentiated compared with well-differentiated tumor (33, 34). Because ionic alterations are important events in malignant transformation, apoptosis, necrosis, and progression through the cell cycle, it is not surprising that successful antineoplastic agents affect IC ions (35, 36). Consistent with our results (Figs. 3 and 4), various antineoplastics (e.g., colchicine, lonidamine, and VP-16) have been reported to elevate [Na] and/or [Ca], (37–40).

For Taxotere, the in vivo injection doses we used for mice were comparable with human clinical doses. Furthermore, human plasma levels of Taxotere after a single loading dose (41) remain above the levels (10 nmol/l) used in our tissue culture studies for ~24 h. Thus, we expect that our tissue culture results (elevation of both [Na], and [Ca], and significant apoptosis) would apply during clinical in vivo tumor studies. The VP-16 tissue culture levels we used were comparable with average human plasma levels during the first 4–6 h after a standard dose (100 mg/m²; Ref. 42).

On the basis of our in vivo and tissue culture studies and in vitro literature reports, the most straightforward explanation for the increased image intensity after chemotherapy is increased [Na]i. We have, however, considered several alternate explanations, which although less likely, still must be investigated: (a) decreased IC T₁ (or change in EC T₁); (b) increased EC binding sites; and (c) increased EC [Na]o. Explanation c seems unlikely,
because even in excitable tissue, where there is a high density of sodium channels and Na⁺/K⁺-ATPase pump sites, there is no evidence of bulk changes in [Na], beyond the unstirred layers around channel pores. For explanation b, one may speculate whether drug-induced processes could alter production of metalloproteinases, affecting the density of EC sodium binding sites. During hypoxia and Na⁺/K⁺-ATPase pump blockade in the heart, multiple quantum sodium MRI studies showed that the

![Image](https://example.com/image.png)

**Fig. 6** Morphological differentiation based on extent of nonviable center. A–C, sodium MR images from a tumor Q with a necrotic center. The noninverted SQ image (C) is uniformly bright, whereas the IR images (A and B) have dark centers. IR image intensity of the annulus increases after drug injection (compare control A with B). The mouse body is delineated in red. The carcinoma (also tumor Q) stained for H&E (lower right panel), showing a narrow rim of viable tissue (V) and a large nonviable center (N). The slim interface (arrows) between viable (V) and necrotic (N) zones shows apoptotic cells (arrow in inset). Bar, 1 mm.
contribution of EC sodium to the increase in sodium image intensity is negligible. As to potential explanation, a reduction in IC $T_1$ (or increase in EC $T_1$) could also contribute to increased image intensity, and one could postulate that large changes in $[\text{Na}]_i$ by themselves could alter IC $T_1$. However, during the intervention of hypoxia or ouabain poisoning, which can dramatically increase $[\text{Na}]_i$, IC $T_1$ values were reported stable (43). Likewise, IC $T_2$ values are stable during pump blockade in the heart (21). Thus, although such alternate hypothesis motivate additional studies, it seems unlikely that such results could alter the diagnostic potential of this measurement.

Absolute quantitation, although a long-term goal of this approach, is confounded by various issues, not the least of which is tissue, cellular, and subcellular compartmentalization. For example, diffusion on a molecular level could result in an exchange of sodium between molecular domains with different $T_1$ values during the recovery from the inversion pulse. This would be the case if the diffusion relaxation time constant were comparable with inversion time. As has been shown in submicron vacuoles (44), this exchange process leads to an averaging of the $T_1$ values and interferes with the ability to distinguish sodium inside and outside the vacuole membrane. However, the size of tumor cells and the limited unidirectional exchange through the polarized cell membranes make substantial exchange between IC and EC compartments during an inversion pulse unlikely (45, 46). Furthermore, measured values of $T_1$ in mammalian IC and EC spaces show primarily single exponential relaxation kinetics. The EC $T_1$ value is longer, consistent with our hypothesis. Sodium localized to regions of the interstitial spaces, where there are more numerous binding sites, do have short $T_1$s (47), but (as above) there is no evidence that they or $T_1$s of the IC contribute a changing component to the IR image (43, 48).

The correlation between apoptosis and IR image intensity is apparent in comparing the histology and images. Tumors with significant fluid spaces or large centers with cellular debris had dark centers on the IR weighted images. In this study, the only tumor with no IR signal had a narrow rim of surviving cells, a large nonviable center with significant fluid, but no significant interface with back-to-back apoptosis. (No chemotherapy was administered for this tumor.) Tumors with bright annuli had sizeable rims of back-to-back apoptosis. Tumors with bright centers had back-to-back apoptosis extending into the central region with minimal nonviable tissue. Although we could not do

![Fig. 7](image_url) Further evidence from images and histology after drug administration. Carcinoma (tumor M) shows a uniformly bright IR image for control, 24 and 48 h (A–C). The outer margin of the IR image is seen to expand by ~100 μm after the 24-h image but prior to the 48-h image. The mouse body is delineated in red. The tumor is visualized at the bottom of the body image, between phantoms. Histology is shown from tumor (M), indicating a small central area of nonviable tissue. In H&E staining (D), the surviving rim of viable cells (V) appears peripheral to a wide region of back-to-back apoptosis (A). The fluorescently end-labeled companion slide (E) indicates a well-demarcated bright region just below the surviving rim, presumably representing the border with the most recently apoptotic cells (illustrated at high power in the inset). A low power field (F) indicates a thick rim of viable tissue (V) and a small nonviable center (N). The broad interface (arrows) between viable (V) and nonviable (N) zones contains back-to-back apoptotic cells (arrows in inset). Bar, 1 mm. The right-hand phantom measures 8 mm in diameter.
a time study of the histological parameters, the results are consistent with the notion that the brightness comes from dense regions of apoptosis and/or early apoptotic processes in the surviving rim. On the basis of the spatial resolution of the image (~1 mm within each slice), the enhanced intensity could be from a spatial broadening of the apoptotic rim, an increased density of apoptotic cells (including early commitment to apoptosis), or both.

Apoptosis is a dynamic process, which ends with complete fragmentation and loss of cell nuclei and most of the other cellular macromolecules. Hence, the absence of a weighted IC sodium MRI signal in late apoptosis can be explained by the loss of polyanions, because the IC space equilibrates its chemical composition with the EC fluid. This prediction is consistent with our finding (Fig. 4) that the average IR intensity increase is smaller at 48 h than at 24 h. A more detailed quantitative analysis is required to correlate specific cell populations with image responses and specific ionic responses with molecular events induced by the chemotherapy.

Because proton MRI, which uses the hydrogen nucleus, is best suited for structural studies and angiography, we have chosen to develop clinical diagnostic approaches using sodium MRI because of the major involvement of sodium in important dynamic processes in the cell, one of which, illustrated here, is the interaction of chemotherapy, cellular apoptosis, and ions. The ease with which sodium images can be coregistered with proton high resolution images is a further advantage of this technique, as is the fact that it does not require costly or potentially toxic reagents, as do traditional SQ sodium MRI or the new techniques used in gene marker or receptor imaging.

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References

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