Metabolic Imaging of Early Tumor Therapy

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Introduction: Many different anti-cancer drugs have been studied by [Dynamic-Contrast-Enhanced] DCE-MRI. The most common pharmacokinetic parameters measure *vascular* properties: K^{trans} [contrast agent (CA) extravasation transfer constant] and v_b [blood volume fraction, proportional to microvessel density]. Thus, many of the therapies studied have been antivascular (1). Not surprisingly, K^{trans} – which depends on vascular permeability, surface area, and [slightly] blood flow – decreases quite quickly after treatment for essentially every such drug tested, and therefore does not discriminate them (1).

Knowledge of early *cellular* metabolic drug consequences should be more informative. The shutter-speed pharmacokinetic analysis of DCE-MRI data allows determination of the cellular *equilibrium* water efflux rate constant, k_{io} [$\equiv 1/\tau_i$], reported proportional to on-going activity [turnover] of the cell membrane Na^+,K^+-ATPase [NKA], perhaps the cell's most vital enzyme (2,3). We report here that k_{io} and v_i [the intracellular volume fraction, proportional to cell density] can provide metabolic and cellular insights into early therapeutic response.

Methods: Since tumors are very heterogeneous, population- and/or ROI-averaging of pharmacokinetic parameters loses information (3). Individual [personalized] lesions must be examined, and mapped with the highest spatial resolution possible. We compare two examples: different therapies on spontaneous *in vivo* human and mouse breast tumors. The human lesion is a grade 2 IDC in a patient who is HER2 positive, BRCA1/BRCA2 mutation negative, and ER/PR positive. The 3T data acquisition/analysis details are given in (3). The mouse model is genetically modified to develop breast tumors driven by activated HER2 signaling and de-regulated c-Myc expression. The 12T data acquisition/analysis details are given in (4).

Results: **Figure 1** shows zoomed axial parametric maps of the right breast human tumor, while **Figure 2** shows analogous maps of the mouse tumor in a right mammary gland. The [a,d], [b,e], and [c,f] panels display K^{trans}, k_{io}, and v_i maps, respectively. The [a-c]/[d-f] panels represent pre-/post-therapy conditions. The nominal voxel sizes are [1.1x1.1x1.4] mm^3 [1.7 µL] and [195x195x750] μm^3 [29 nL] for human/mouse. The Fig. 1d-f human maps were obtained 17 days after one IV infusion of 272 mg trastuzumab/140 mg paclitaxel. The Fig. 2d-f murine maps were obtained after 10 days of daily 5 mg/kg OP449 IP injection.

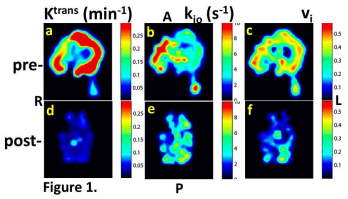

Figure 1.

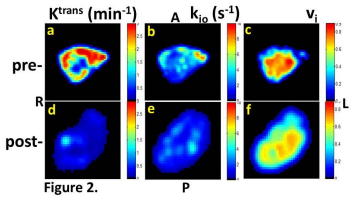

Figure 2.

Though the pre-therapy tumors are different sizes [2 cm and 0.5 cm diameters; human/mouse], they have some phenotypic similarities. First, the pre-therapy K^{trans} maps [Figs. 1a, 2a] both show rim-enhancement: rim K^{trans} is larger. Second, the tumor ROI-averaged K^{trans} is decreased dramatically [79% and 71%; human/mouse] after therapy [Figs. 1d/2d] – though they have different pre-therapy values [0.2 min^{-1}/0.7 min^{-1}]. Even though these therapies are quite different, a K^{trans} decrease is very common, as we have seen above. Third, the k_{io} maps report greater tumor rim metabolic activity [NKA turnover], but not in all elevated K^{trans} regions. Therapy generally reduces k_{io} in each tumor.

The v_i parameter [$\equiv 1 - v_e$] is the cell density•volume product, ρ•V: the voxel cell number density times the *mean* cell volume (3). Since the human voxels average ~500,000 (3) and the murine voxels ~9000 cells, it is a reasonable assumption that V doesn't change greatly [a conservatively large cell diameter is 15 µm (3)]. If this is valid, v_i variation is dominated by ρ variation. The v_i maps distinguish the lesions: the human tumor has greater rim ρ, while the mouse tumor has a greater core ρ. Furthermore, therapy greatly diminishes the human tumor v_i but less so for the mouse.

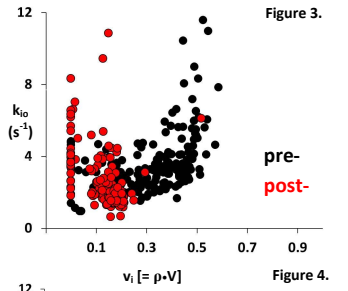

Figure 3.

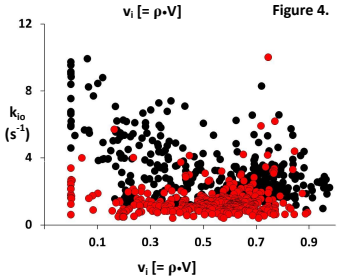

Figure 4.

Figures 3/4 show pixel-by-pixel k_{io} *vs.* v_i scatter plots for the human/murine tumors: to further distinguish them and the therapeutic responses. The black/red points represent the pre-/post-therapy conditions. By definition, k_{io} is ρ-independent, and it depends on only $V^{-1/3}$ (3). The Fig. 3/4 plots show that the k_{io} and v_i parameters are effectively independent: *i.e.*, not numerically correlated by the analytical DCE-MRI data fitting, which would produce sloped clusters. The Fig. 3 vertically oriented pre-therapy cluster corresponds to the high human tumor lateral [right, R] rim activity [Fig. 1b]: varying NKA activity with essentially invariant v_i. Otherwise, there is no general cluster orientation. These observations indicate k_{io} is dominated by cell membrane water permeability and/or that V does not vary significantly (3). In both plots, therapy reduces the k_{io} values, but more-so for the murine tumor. The v_i parameter is reduced in the human tumor: if anything, non-zero v_i is slightly increased in the mouse lesion.

Discussion: These results give some insights into the therapeutic mechanisms. Paclitaxel is a well-known cytotoxic drug (antimitotic; interfering with microtubule formation). Trastuzamab is a monoclonal antibody molecule targeted to the HER2 receptor. It surely exerts some therapeutic effect by interfering with HER2-facilitated growth signaling, but probably also by recruitment of cell-mediated cytotoxicity. Certainly the neoadjuvant paclitaxel/trastuzamab cocktail is intended to be cytotoxic. The considerable reduction of v_i [probably ρ], Figs. 1c,f/3, in the human tumor rim signifies substantial cell destruction.

The drug OP449, on the other hand, exemplifies a purely targeted therapy. It is a peptide mimetic that re-activates the crucial tumor suppressor enzyme PP2A [protein phosphatase 2A]. It does this by antagonizing the SET oncoprotein that is the cancer cell's PP2A inhibitor (4-6). In this sense, it re-normalizes the malignant cell. The Figs. 2b,c,e,f/4 results are consistent with this mechanism. It is possible to considerably reduce tumor NKA turnover, without much [if any] cell destruction in the lesion that remains.

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