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Experimental MR imaging

Dynamic contrast-enhanced magnetic resonance imaging of tumor interstitial fluid pressure

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ABSTRACT

Background and purpose: High interstitial fluid pressure (IFP) in the primary tumor has been shown to promote metastasis in melanoma xenografts and to predict for poor survival in cervical cancer patients. The potential usefulness of gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA)-based dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) for assessing tumor IFP noninvasively was investigated in the present study.

Materials and methods: A-07 and R-18 melanoma xenografts with and without necrotic regions were subjected to DCE-MRI and subsequent measurement of IFP. Tumor images of $E{\cdot}F$ (E is the initial extraction fraction of Gd-DTPA and F is blood perfusion) and λ (λ is proportional to extracellular volume fraction) were produced by Kety analysis of DCE-MRI series.

Results: In tumors without necrosis, significant inverse correlations were found between $E{\cdot}F$ and IFP, both for A-07 and R-18 tumors, and between λ and IFP for A-07 tumors. Significant correlations beween $E{\cdot}F$ and IFP or between λ and IFP could not be detected for tumors with necrotic regions.

Conclusions: DCE-MRI may be developed to be a useful noninvasive method for assessing IFP in tumors without necrosis. This possibility warrants further studies involving several physiologically different experimental tumor lines, as well as human tumors.

Tumors develop elevated interstitial fluid pressure (IFP) during growth, mainly because of increased fluid permeability of the microvascular network and lack of functioning lymphatic vessels [1–3]. Differences in IFP among tumors result primarily from differences in resistance to microvascular blood flow, interstitial hydraulic conductivity, and interstitial matrix elasticity [2]. Elevated IFP may lead to poor tumor uptake of macromolecular therapeutic agents and, hence, resistance to some forms of immunotherapy and gene therapy [3]. Studies of melanoma xenografts have suggested that high IFP promotes pulmonary and lymph node metastasis [4]. Investigations of cervical cancer patients treated with radiation therapy have shown that high IFP is correlated with high likelihood of pelvic recurrence and distant metastases [5]. Moreover, long-term studies of cervical cancer patients have suggested that high tumor IFP may have a stronger prognostic impact for survival than tumor hypoxia [6].

A few investigators have attempted to use magnetic resonance imaging for noninvasive assessment of the IFP of tumors [7–9]. Lyng et al. [7] did not detect significant correlations between IFP and T_1 or T_2 relaxation rates in a study of melanoma xenografts. By using a protocol based on slow infusion of gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA), Hassid et al. [8] showed that parametric images of the steady-state concentration of Gd-DTPA reflected the spatial distribution of IFP in non-small-cell carcinoma xenografts and breast carcinoma xenografts, but correlations between IFP and steady-state Gd-DTPA concentration were not reported. Haider et al. [9] subjected patients with cervical cancer to dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and measurement of tumor IFP, and found significant correlations between IFP and DCE-MRI-derived parameters. However, the correlations were weak, possibly because the DCE-MRI parameters were derived from a single region of interest encompassing the tumor rather than from voxel-by-voxel parametric images [9].

The potential usefulness of Gd-DTPA-based DCE-MRI for characterizing the physiological microenvironment of tumors is currently being evaluated in our laboratory [10–12]. In previous preclinical studies, we have shown that highly reproducible images of $E{\cdot}F$ (E is the initial extraction fraction of Gd-DTPA and F is blood perfusion) and λ (λ is proportional to extracellular volume fraction) can be obtained by subjecting DCE-MRI series to Kety analysis [10,11]. The purpose of the study reported here was to investigate whether high-resolution DCE-MRI-derived parametric images may provide information on the IFP of tumors. Because tumors with high IFP may have poor blood perfusion due to high resistance

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against blood flow and/or low interstitial hydraulic conductivity due to a low extracellular volume fraction [2], we hypothesized that tumors with high IFP would show low $E{\cdot}F$ values and/or low λ values. To test this hypothesis, human tumor xenografts differing substantially in blood perfusion, extracellular volume fraction, and fraction of necrotic tissue were subjected to DCE-MRI and subsequent measurement of IFP.

Materials and methods

Tumor models

A-07 and R-18 human amelanotic melanoma xenografts, initiated by inoculating $\sim 3.5 \times 10^5$ cells intradermally in the flank of adult (8–10 weeks of age) female BALB/c *nu/nu* mice, were used as tumor models [13]. The mice were stabled under specific pathogen-free conditions and were given sterilized food and tap water *ad libitum*. Tumors without necrotic regions were obtained by keeping the host mice at a temperature of 24–26 °C and a humidity of 30–50%. To obtain tumors with necrotic regions, the host mice were kept at a temperature of 30–32 °C and a humidity of 60–70%. The experiments were initiated when the tumors had grown to a volume of 200–600 mm^3. Fourteen A-07 tumors without necrosis, 13 A-07 tumors with necrotic regions, 13 R-18 tumors without necrosis, and 12 R-18 tumors with necrotic regions were included in the study. The tumors were subjected to measurement of IFP immediately after DCE-MRI. Animal care and experimental procedures were approved by the Institutional Committee on Research Animal Care and were performed in accordance with the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing, and Education (New York Academy of Sciences, New York, NY, USA).

Anesthesia

DCE-MRI and measurement of IFP were carried out with anesthetized mice. Fentanyl citrate (Janssen Pharmaceutica, Beerse, Belgium), fluanisone (Janssen Pharmaceutica), and midazolam (Hoffmann-La Roche, Basel, Switzerland) were administered i.p. in doses of 0.63 mg/kg, 20 mg/kg, and 10 mg/kg, respectively. By using the ^{86}Rb uptake method, it was verified that the anesthesia did not alter tumor blood perfusion significantly [i.e., the ^{86}Rb uptake (mean ± SE; n = 8–10) in units of% of injected $^{86}Rb \times g^{-1}$ of tumor tissue $\times$ g of mouse body weight was 57 ± 5 (A-07) and 39 ± 4 (R-18) in anesthetized mice and 54 ± 4 (A-07) and 42 ± 6 (R-18) in unanesthetized mice].

DCE-MRI

The DCE-MRI experiments and the subsequent image processing and analysis were carried out as described in detail previously [14]. Briefly, a 24-G neoflon connected to syringe by a polyethylene tubing was inserted in the tail vein of tumor-bearing mice, and Gd-DTPA (Schering, Berlin, Germany) was administered manually in a bolus dose of 0.3 mmol/kg after the mice had been positioned in the magnet. T_1-weighted DCE-MRI was performed at a spatial resolution of $0.31 \times 0.31 \times 2.0$ mm^3 and a time resolution of 14 s by using a 1.5-T whole-body scanner (Signa; General Electric, Milwaukee, WI, USA) and a slotted tube resonator transceiver coil constructed for mice. Tumor images were analyzed on a voxel-by-voxel basis by using software developed in IDL (Interactive Data Language, Boulder, CO, USA). Gd-DTPA concentrations were calculated from signal intensities by using the method of Hittmair et al. [15]. Plots of Gd-DTPA concentration *versus* time were generated, and the modified Kety equation [16],

$$C_t(T) = E \cdot F \cdot \rho \cdot \int_0^T C_a(t) \cdot e^{-E \cdot F \cdot \rho (T-t)\lambda} dt,$$

was fitted to the plots, where $C_t(T)$ is the Gd-DTPA concentration in the tumor tissue at time T, E is the initial extraction fraction of Gd-DTPA, F is the perfusion per unit tumor weight, ρ is the density of the tumor tissue (1 g/ml), $C_a(t)$ is the arterial input function, and λ is a parameter that is proportional to the extracellular volume fraction of the tumor tissue. The arterial input function was determined as described elsewhere [10]. Numerical values of $E{\cdot}F$ [in units of ml/(g min)] and λ were determined for each voxel from the best curve fit. Images of $E{\cdot}F$ and λ were generated by using SigmaPlot software (SPSS Science, Chicago, IL, USA). $E{\cdot}F$ and λ are related to the parameters of the commonly used Tofts pharmacokinetic model (K^{trans}, the volume transfer constant of Gd-DTPA, and υ_e, the extracellular volume fraction of the imaged tissue) by the following expressions: $E{\cdot}F = K^{trans}/[\rho \cdot (1 - Hct)]$ and $\lambda = \upsilon_e / (1 - Hct)$, where Hct is the hematocrit [16].

Tumor necrosis

Necrotic tumor regions were identified in λ images by using a procedure described in detail elsewhere [17,18]. This procedure is based on three observations: (a) voxels located in small necroses and in the periphery of large necrotic regions show Gd-DTPA concentration *versus* time curves that increase nearly linearly with time owing to diffusion of Gd-DTPA from the viable tissue into the necrotic tissue, giving rise to unphysiologically high λ values, usually $\lambda > 1000$, (b) voxels located in the center of large necrotic regions show no uptake of Gd-DTPA (i.e., Gd-DTPA concentration *versus* time curves that do not increase with time), giving rise to unphysiologically low λ values, usually $\lambda < 0.01$, and (c) voxels located in viable tissue show Gd-DTPA concentration *versus* time curves giving rise to physiological λ values, usually $0.05 < \lambda < 1.0$, depending on the cell density and, hence, the tumor line. Therefore, voxels showing higher λ values than the highest values observed for viable tissue (λ_{high}) and the voxels showing lower λ values than the lowest values observed for viable tissue (λ_{low}) were considered to represent necrotic tissue. The fraction of voxels showing λ values $> \lambda_{high}$ or λ values $< \lambda_{low}$ has been shown to correspond well with the fraction of necrotic tissue as determined by analysis of histological preparations [17,18].

IFP

IFP was measured twice in the center of the tumors by using a Millar SPC 320 cathether equipped with a 2F Mikro-Tip tranceducer with diameter 0.66 mm (Millar Instruments, Houston, TX, USA) [19]. The cathether was connected to a computer via a Millar TC-510 control unit and a model 13-66150-50 preamplifier (Gould Instruments, Cleveland, OH, USA). Data acquisition was performed by using LabVIEW software (National Instruments, Austin, TX, USA).

Statistical analysis

The Pearson product moment correlation test was used to search for correlations between variables. Statistical comparisons of data sets were carried out by using the Student's t-test when the data sets complied with the conditions of normality and equal variance. Under other conditions, comparisons were carried out by nonparametric analysis using the Mann–Whitney rank sum test. The Kolmogorov–Smirnov method was used to test for normality. Probability values of $P < 0.05$, determined by using the Bonferroni correction for multiple comparisons, were considered