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MRI hypoxic imaging

Differentiation between hypoxic and non-hypoxic experimental tumors by dynamic contrast-enhanced magnetic resonance imaging

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ABSTRACT

Background and purpose: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has been suggested to be a useful method for detecting tumor hypoxia. In this study, we investigated whether DCE-MRI can differentiate between hypoxic and non-hypoxic experimental tumors.

Materials and methods: Three tumor models with hypoxic tissue and three tumor models without hypoxic tissue were subjected to DCE-MRI. Parametric images of K^{trans} (the volume transfer constant of Gd-DTPA) and v_e (the fractional distribution volume of Gd-DTPA) were produced by pharmacokinetic analysis of the DCE-MRI series. Tumor oxygenation status was assessed by using a radiobiological assay and a pimonidazole-based immunohistochemical assay. Tumor response to fractionated irradiation (six fractions of 2 Gy in 60 h) was measured *in vitro* by using a clonogenic assay.

Results: Tumors with hypoxic regions were more resistant to radiation treatment than were tumors without hypoxia. K^{trans} was significantly higher for radiation sensitive tumors without hypoxia than for radiation resistant tumors with hypoxic regions, whereas v_e did not differ significantly between non-hypoxic and hypoxic tumors.

Conclusion: This study supports the clinical attempts to establish DCE-MRI as a noninvasive method for providing useful biomarkers for personalized radiation therapy.

Regions with hypoxic tissue (pO_2 < 10 mm Hg) are a characteristic feature of many tumors [1]. Tumor hypoxia may cause resistance to treatment and promote metastatic spread [2]. Studies of several histological types of cancer have suggested that patients with hypoxic tumors may benefit from particularly aggressive treatment [3]. A noninvasive method for detecting tumors with significant hypoxia is therefore highly required [4–6]. Preliminary preclinical and clinical studies have suggested that valid information on the oxygen tension in tumors may be obtained by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) [7,8].

The potential of gadolinium diethylene-triamine penta-acetic acid (Gd-DTPA)-based DCE-MRI in detecting hypoxic regions in tumors is currently being evaluated in our laboratory by using xenografted human tumors as preclinical models of human cancer [9–13]. In these studies, parametric images of K^{trans} and v_e are produced by subjecting DCE-MRI series to pharmacokinetic analysis using the modified Kety model developed by Tofts et al. [14]. The studies carried out thus far have shown that our experimental procedure produces highly reproducible parametric images [9,10] and that the parametric images may provide valuable information on blood perfusion, extracellular volume fraction, and the extent of hypoxia in tumors [11–13].

In the present study, the potential of DCE-MRI in providing information on the oxygenation status of tumors was investigated further by subjecting tumors with hypoxic tissue and tumors without hypoxic tissue to DCE-MRI. Tumors of two human melanoma xenograft lines and two transplantation sites were included in the study. The study was based on earlier investigations in several laboratories having shown that the microvascular network and the extent of hypoxia in experimental tumors are influenced significantly by the angiogenic profile of the tumor cells and the vascularity of the transplantation site [12,15–17]. The main purpose of the study was to investigate whether DCE-MRI has the potential to distinguish tumors with hypoxic tissue from tumors without hypoxia.

Materials and methods

Tumor models

A-07 and R-18 human melanoma xenografts growing in adult female BALB/c *nu/nu* mice were used as tumor models [18]. Tumors were initiated from cells cultured in RPMI-1640 medium supplemented with 13% bovine calf serum, 250 mg/l penicillin, and 50 mg/l streptomycin. Approximately 3.5×10^5 cells in 10 μl of Hanks' balanced salt solution (HBSS) were inoculated intrader-

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mally (i.d.) or intramuscularly (i.m.) in the leg. Tumors with volumes of 75–150 mm^3 (small tumors) or 300–600 mm^3 (large tumors) were included in experiments. Three tumor models without hypoxia (small A-07 i.d., small A-07 i.m., and small R-18 i.d.) and three tumor models with hypoxic tissue (large A-07 i.d., large A-07 i.m., and large R-18 i.d.) were studied. Tumor irradiation and DCE-MRI were carried out with mice anesthetized with fentanyl citrate (Janssen Pharmaceutica, Beerse, Belgium), fluanisone (Janssen Pharmaceutica), and midazolam (Hoffmann-La Roche, Basel, Switzerland) in doses of 0.63, 20, and 10 mg/kg, respectively. Animal care and experimental procedures were 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).

Tumor irradiation and cell survival

Tumors were irradiated under air-breathing or hypoxic conditions at a dose rate of 5.1 Gy/min, using an X-ray unit operated at 220 kV, 19–20 mA, and with 0.5-mm Cu filtration. Hypoxic tumors were obtained by euthanizing the host mice 5 min before the radiation exposure. Cell surviving fractions were measured *in vitro*. The tumors were given single dose irradiation or fractionated radiation treatment (six fractions of 2 Gy in 60 h). They were resected immediately after the radiation exposure, minced in cold HBSS, and treated with an enzyme solution (0.2% collagenase, 0.05% Pronase, and 0.02% DNase) at 37 °C for 2 h. Tumor volume was measured prior to the irradiation, and the cell yield was determined as the total number of trypan blue–negative cells divided by the tumor volume. Trypan blue–negative cells were plated in 25-cm^2 tissue culture flasks and incubated at 37 °C for 14 days for colony formation [19]. Cell surviving fractions were calculated from the cell yield, the number of cells seeded, and the number of colonies counted, corrected for the mean cell yield and the mean plating efficiency of the cells of six untreated control tumors, i.e., the cell surviving fractions of tumors given fractionated radiation treatment were measured relative to the number of clonogenic cells in the tumors before the first radiation exposure [20].

DCE-MRI

DCE-MRI was carried out as described earlier [13]. Briefly, Gd-DTPA (Schering, Berlin, Germany) was administered in a bolus dose of 0.3 mmol/kg. T_1-weighted images (TR = 200 ms, TE = 3.2 ms, and $\alpha_{T1} = 80°$) were recorded 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) and a slotted tube resonator transceiver coil constructed for mice. Two calibration tubes, one with 0.5 mmol/l Gd-DTPA in 0.9% saline and the other with 0.9% saline only, were placed adjacent to the mice in the coil. The tumors were imaged axially in a single section through the tumor center by using an image matrix of 256×128, a field of view of 8×4 cm^2, and one excitation. Two proton density images (TR = 900 ms, TE = 3.2 ms, and $\alpha_{PD} = 20°$) and three T_1-weighted images were acquired before Gd-DTPA was administered, and T_1-weighted images were recorded for 15 min after the administration of Gd-DTPA. Gd-DTPA concentrations were calculated from signal intensities by using the method of Hittmair et al. [21]. The DCE-MRI series were analyzed on a voxel-by-voxel basis by using the arterial input function of Benjaminsen et al. [9] and the Tofts pharmacokinetic model [14]. Parametric images of K^{trans} (the volume transfer constant of Gd-DTPA) and v_e (the fractional distribution volume of Gd-DTPA) were generated by using the SigmaPlot software (SPSS Science, Chicago, IL, USA). Median values of K^{trans} and v_e were calculated for the viable tissue of the tumors by excluding the voxels in necroses, using a procedure described elsewhere [15].

Histological examinations

Pimonidazole [1-[(2-hydroxy-3-piperidinyl)-propyl]-2-nitroimidazole] was used as a marker of tumor hypoxia [22]. The tumors were fixed in phosphate-buffered 4% paraformaldehyde immediately after the DCE-MRI, and histological sections were prepared by using standard procedures. Immunohistochemistry was performed by using an avidin–biotin peroxidase-based staining method [19]. An anti-pimonidazole rabbit polyclonal antibody (gift from Professor Raleigh, Department of Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, NC) was used as primary antibody. Diaminobenzidine was used as chromogen, and hematoxylin was used for counterstaining. Three cross-sections were examined for each tumor.

Statistical analysis

Experimental data are presented as arithmetic mean ± SE unless otherwise stated. Statistical analyses were carried out by one-way ANOVA followed by the Student Newman–Keuls test. The method of Kolmogorov–Smirnov was used to test for normality. Probability values of $P < 0.05$ were considered significant. The statistical analysis was carried out by using the SigmaStat statistical software (SPSS Science).

Results

Tumors of all six models were exposed to single graded radiation doses under air-breathing or hypoxic conditions to assess the radiation sensitivity of the tumor cells and the oxygenation status of the tumors. The D_0 values of the cell survival curves were found to be 0.89 ± 0.08 Gy (air-breathing conditions) and 2.51 ± 0.10 Gy (hypoxic conditions) for small A-07 i.d. tumors, 2.52 ± 0.11 Gy (air-breathing conditions) and 2.56 ± 0.13 Gy (hypoxic conditions) for large A-07 i.d. tumors, 0.88 ± 0.09 Gy (air-breathing conditions) and 2.54 ± 0.12 Gy (hypoxic conditions) for small A-07 i.m. tumors, 2.49 ± 0.13 Gy (air-breathing conditions) and 2.53 ± 0.12 Gy (hypoxic conditions) for large A-07 i.m. tumors, 1.05 ± 0.10 Gy (air-breathing conditions) and 2.99 ± 0.11 Gy (hypoxic conditions) for small R-18 i.d. tumors, and 3.02 ± 0.10 Gy (air-breathing conditions) and 2.97 ± 0.12 Gy (hypoxic conditions) for large R-18 i.d. tumors (Fig. 1). The survival curves of the tumors irradiated under air-breathing conditions were either steeper than (small tumors) or parallel to (large tumors) those of the tumors irradiated under hypoxic conditions. The oxygen enhancement ratios of the small tumors were calculated from the slopes of the survival curves to be 2.82 ± 0.28 (A-07 i.d.), 2.89 ± 0.33 (A-07 i.m.), and 2.85 ± 0.29 (R-18 i.d.). The survival curves of the small tumors were thus consistent with no or insignificant hypoxia in the tumors. The fractions of hypoxic cells of the large tumors were calculated from the vertical displacement of the survival curves to be 8 ± 3% (A-07 i.d.), 25 ± 5% (A-07 i.m.), and 58 ± 10% (R-18 i.d.).

Response to fractionated irradiation was studied by irradiating tumors in air-breathing mice with six fractions of 2 Gy in 60 h. The fraction of surviving cells was significantly higher for the three tumor models with hypoxic cells than for the three tumor models without hypoxia (Fig. 2; $P < 0.001$). Furthermore, the surviving fraction was significantly higher for large R-18 i.d. tumors than for large A-07 i.m. tumors ($P < 0.01$) and significantly higher for large A-07 i.m. tumors than for large A-07 i.d. tumors ($P < 0.05$), i.e., it decreased with decreasing fraction of hypoxic cells.

Tumors of all models were subjected to DCE-MRI to investigate whether K^{trans} and v_e images can differentiate between tumors