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BIOLOGY CONTRIBUTION

ASSESSMENT OF HYPOXIA IN HUMAN CERVICAL CARCINOMA XENOGRAFTS BY DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING

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Purpose: **Patients with advanced cervical cancer and highly hypoxic primary tumors show increased frequency of locoregional treatment failure and poor disease-free and overall survival rates. The potential usefulness of gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA)–based dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in assessing tumor hypoxia noninvasively was investigated in the present preclinical study.**
Methods and Materials: **CK-160 and TS-415 human cervical carcinoma xenografts transplanted intramuscularly (i.m.) or subcutaneously (s.c.) in BALB/c *nu/nu* mice were subjected to DCE-MRI and measurement of fraction of radiobiologically hypoxic cells. Tumor images of K^{trans} (the volume transfer constant of Gd-DTPA) and v_e (the extracellular volume fraction of the imaged tissue) were produced by pharmacokinetic analysis of the DCE-MRI data. Fraction of radiobiologically hypoxic cells was measured by using the paired survival curve method.**
Results: **Fraction of radiobiologically hypoxic cells differed significantly among the four tumor groups. The mean values ± SE were determined to be 44% ± 7% (i.m. CK-160), 77% ± 10% (s.c. CK-160), 23% ± 5% (i.m. TS-415), and 52% ± 6% (s.c. TS-415). The four tumor groups differed significantly also in K^{trans}, and there was an unambiguous inverse relationship between K^{trans} and fraction of radiobiologically hypoxic cells. On the other hand, significant differences among the groups in v_e could not be detected.**
Conclusions: **The study supports the clinical development of DCE-MRI as a method for assessing the extent of hypoxia in carcinoma of the cervix.** © **2009 Elsevier Inc.**

Cervical carcinoma, DCE-MRI, Hypoxia, Radiation sensitivity, Xenografts.

INTRODUCTION

Advanced squamous cell carcinoma of the uterine cervix is primarily treated with radiation alone or radiation in combination with surgery and/or chemotherapy (1). In the 1960s, hypoxia was identified as a characteristic feature of cervical carcinoma that could cause resistance to radiation therapy (2). Recent studies have confirmed these initial observations (3–6) and have also shown that hypoxia may be an adverse prognostic factor in patients given surgery as primary treatment (3). Moreover, extensive hypoxia in the primary tumor has been shown to be associated with biologic aggressiveness, invasive growth, and increased metastatic propensity (7, 8). Thus, cervical carcinoma patients with highly hypoxic tumors show increased frequency of locoregional treatment failure and poor disease-free and overall survival rates (9, 10), and these patients may therefore benefit from particularly aggressive treatment. Consequently, a noninvasive diagnostic method for assessing the extent of hypoxia in cervical tumors is needed.

Preclinical and clinical studies have suggested that gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA)–based dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) may be a useful noninvasive method for characterizing the physiologic microenvironment of tumors (11). A DCE-MRI–based method is particularly attractive in cervical carcinoma patients because DCE-MRI is an established and commonly used method for depicting the primary tumor in these patients (12). Moreover, initial investigations of the prognostic value of DCE-MRI in carcinoma of the cervix have given promising results. Mayr *et al* (13, 14) observed that a high relative signal intensity in the primary tumor was associated with a high local control rate in patients given radiation therapy. High levels of contrast enhancement could be a result of efficient blood perfusion and thus reflect good oxygenation and hence increased radioresponsiveness. Alternatively, high levels of contrast enhancement could be a result of large extracellular volume fractions and thus reflect low numbers of clonogenic cells and hence increased radiocurability.

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There has been an increasing interest in using DCE-MRI for characterizing the physiologic microenvironment of cervical tumors (15–19). Correlations have been found between DCE-MRI–derived parameters and microvascular density, amount of interstitial fibrous tissue, tumor cell density, and extracellular volume fraction as assessed by histologic examinations of the imaged tissue (15–17) as well as tumor oxygenation as assessed by polarographic measurements of tissue pO_2 (17–19). However, the correlations are generally weak, perhaps because unsuitable parameters were derived from the DCE-MRI series (*i.e.,* parameters calculated from signal intensity curves without the use of pharmacokinetic models or by the use of inadequate models) or because the DCE-MRI was performed under suboptimal conditions (*i.e.,* inadequate temporal and spatial resolution, low signal-to-noise ratio, and/or significant motion artifacts). High-quality preclinical studies are therefore needed to establish whether DCE-MRI has the potential to provide clinically useful surrogate parameters for the extent of hypoxia in cervical carcinoma.

The potential usefulness of Gd-DTPA-based DCE-MRI in assessing fraction of hypoxic cells in human tumor xenografts is currently being evaluated in our laboratory. Our studies are based on the hypothesis that tumor hypoxia is the result of an imbalance between oxygen supply and oxygen consumption (20). The oxygen supply is determined primarily by the blood perfusion, and the oxygen consumption is determined primarily by the respiratory activity of the tissue and, hence, the cell density (21). Hypoxic tissue is therefore expected to be found in tumor regions with poor blood perfusion and/or low extracellular volume fraction. We have already shown that DCE-MRI–derived parametric images may provide information on the extent of hypoxia in human melanoma xenografts (22, 23). The purpose of the work reported here was to investigate the feasibility of DCE-MRI in assessing the extent of hypoxia in human cervical carcinoma xenografts. Tumors of two xenograft lines differing significantly in histologic appearance, cellular radiation sensitivity, and fraction of radiobiologically hypoxic cells were included in the study.

METHODS AND MATERIALS

Mice and tumors

CK-160 and TS-415 human cervical carcinoma xenografts growing in adult (8-12 weeks of age) female BALB/c *nu/nu* mice were used as experimental tumor models. Tumors were initiated from established cell lines cultured in RPMI-1640 (25 mmol/l HEPES and L-glutamine) supplemented with 13% bovine calf serum, 250 mg/l penicillin, and 50 mg/l streptomycin. The CK-160 line was established from a pelvic lymph node metastasis of a 65-year-old woman who had developed a highly invasive, well-differentiated (histologic Grade I), keratinizing primary tumor. The TS-415 line was derived from a pelvic lymph node metastasis of a 45-year-old woman who had developed a highly invasive, poorly differentiated (histologic Grade III), nonkeratinizing primary tumor.

The mice were kept under specific pathogen-free conditions and were given sterilized food and tap water *ad libitum*. Approximately 5×10^5 cells in 10 μl of Hanks' balanced salt solution were inoculated in the gastrocnemius muscle to produce intramuscular (i.m.) tumors or in the subcutis far back on the flank to produce subcutaneous (s.c.) tumors. Experiments were initiated when the tumors had grown to a volume of 200 to 600 mm^3. Tumor volume (V) was calculated as $V = \pi/6 \times a \times b^2$, where a is the longer and b is the shorter of two perpendicular diameters. Both DCE-MRI and tumor irradiation 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. 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).

DCE-MRI

The DCE-MRI technique was carried out on 15 i.m. CK-160 tumors, 16 s.c. CK-160 tumors, 15 i.m. TS-415 tumors, and 14 s.c. TS-415 tumors by using a procedure described in detail previously (23). Briefly, a 24-G neoflon connected to a syringe by a polyethylene tubing was inserted in the tail vein of tumor-bearing mice, and Gd-DTPA (Schering, Berlin, Germany) was administered in a bolus dose of 5.0 ml/kg (0.3 mmol/kg) after the mice had been positioned in the magnet. Imaging was performed at a spatial resolution of $0.23 \times 0.47 \times 2.0$ 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 13-cm-long, cylindrical, slotted tube resonator transceiver mouse coil with a diameter of 40 mm. 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 a scan thickness of 2 mm, a number of excitations of 1, an image matrix of 256×64, and a field of view of 6×3 cm^2. Two types of spoiled gradient recalled images were recorded: proton density images with repetition time TR = 900 ms, echo time TE = 3.2 ms, and flip angle $\alpha = 20°$, and T_1-weighted images with TR = 200 ms, TE = 3.2 ms, and $\alpha = 80°$. Two proton density images 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.

The Gd-DTPA concentrations were calculated from signal intensities as described by Hittmair *et al* (24), and the DCE-MRI series were analyzed on a voxel-by-voxel basis by using the arterial input function established by Benjaminsen *et al* (25) and the pharmacokinetic model developed by Tofts *et al* (26). Two parameters were determined for each voxel: K^{trans}, the volume transfer constant of Gd-DTPA, and v_e, the extracellular volume fraction of the imaged tissue. Images of K^{trans} and v_e were generated by using the SigmaPlot software (SPSS Inc., Chicago, IL).

Fraction of radiobiologically hypoxic cells

Intramuscular CK-160 tumors, s.c. CK-160 tumors, i.m. TS-415 tumors, and s.c. TS-415 tumors were irradiated at a dose rate of 5.1 Gy/min by using a Siemens Stabilipan X-ray unit, operated at 220 kV, 19-20 mA, and with 0.5-mm copper filtration (27). Hypoxic tumors were obtained by occluding the blood supply with a clamp 5 min before irradiation.

Tumor cell survival was measured *in vitro* by using a plastic surface colony assay (28). Briefly, the tumors were resected