Pharmacokinetic Analysis of Malignant Pleural Mesothelioma—Initial Results of Tumor Microcirculation and its Correlation to Microvessel Density (CD-34)[1]

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Rationale and Objectives. Malignant mesothelioma (MM) of the pleura is an aggressive and often fatal neoplasm. Because MM frequently demonstrates marked angiogenesis, it may be responsive to antiangiogenic therapy, but effective methods for selecting and monitoring of patients are further needed. We employed dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and quantitative immunohistochemistry (IHC) to characterize the microvascularity of MM using both a physiologic and ultrastructural method.

Materials and Methods. Nineteen patients diagnosed with MM were enrolled and DCE-MRI was performed before antiangiogenic treatment. For each patient, tumor regions were characterized by their DCE-MRI–derived pharmacokinetic parameters (Amp, k_{ep}, k_{el}), which were also compared to those of normal tissue (aorta, liver, spleen, and muscle). In addition, quantitative IHC of representative samples was performed with CD-34 staining to compare the calculated microvessel density (MVD) results with DCE-MRI results.

Results. MM demonstrated markedly abnormal pharmacokinetic properties compared with normal tissues. Among the parameters tested, Amp was significantly different in MM ($P \leq .001$) compared to normal organs. Despite the observation that the MVD of mesotheliomas in this series was high compared to other tumors, DCE-MRI pharmacokinetic parameters had a moderately positive correlation with MVD ($r = 0.5$).

Conclusions. DCE-MRI and IHC can be used in patients with MM to visualize tumor microvascularity and to characterize tumor heterogeneity. DCE-MRI and IHC results positively correlated, though moderately, but these two methods present as essential tumor biomarkers. This multimodal characterization may be useful in selecting possible tumor subtypes that would benefit from antiangiogenic therapy.

Key Words. Angiogenesis; malignant mesothelioma; dynamic contrast enhanced MRI; pharmacokinetic analysis; microvascular density; CD-34.

Malignant epithelioid mesothelioma (MM) is one of the three main types of mesotheliomas. It is an aggressive tumor of the pleura and often fatal. Most, if not all, MMs are associated with prior exposure to asbestos (1,2). Although this epithelioid type has the best prognosis, standard treatment regimens, including surgery, chemotherapy, and radiation are often unsuccessful (median survival 9 months). Nevertheless, long-term survivors have been reported, and new chemotherapy and multimodal regimens show promising results (3).

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Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a new biomarker imaging technique and a method of characterizing tumor's neovascularity in situ (4,5) as well as assessing the effects of therapy on tumor angiogenesis (4–7). DCE-MRI is a noninvasive procedure in which sequential images with high spatial and temporal resolution are obtained to observe the kinetics of contrast media arrival and clearance through the tumor microcirculation and adjacent tissues. The contrast agent enhancement pattern enables visualization, characterization, and quantification of lesion microcirculation (4,8–10). DCE-MRI parameters allowed the prediction of response to therapy in some cases (8,11); however, not all tumors have shown strong correlations between their DCE-MRI patterns and their angiogenic expression.

Quantitative immunohistochemistry (IHC) provides an objective assessment of microvascular ultrastructure. High-affinity stains to endothelial cells allow the semiquantitative assessment of the fraction of the tumor occupied by endothelial cells and thus is an indirect measure of tumor angiogenesis. Quantitative methods allow IHC to be more objective than it has been in the past.

In vivo and ex vivo tumor characterization are likely to yield different but complementary information about tumors and their potential response to therapy. The purpose of this feasibility study was therefore to assess the ultrastructural and functional properties of the microvasculature of MM and determine the degree of correlation between these two modalities for assessing angiogenesis in MM.

MATERIAL AND METHODS

Patients and Diagnostic Evaluation

Tumor staging and classification was performed according to the Butchart malignant pleural mesothelioma classification (12,13) and World Health Organization/International Union Against Cancer criteria. A total of 19 patients (17 male, 2 female, age range 53–77 years, mean 62.5) diagnosed with Stages II ($n = 9$) and IV ($n = 10$) MM were enrolled in a clinical study before chemotherapy; consent to undergo additional imaging procedures was obtained. Final diagnosis was established by immunohistopathology (2). All reported patients were enrolled under an investigational protocol that was approved by the institutional review board of the university clinics. Written informed consent was obtained from all patients after explaining the full procedure and they had full rights to refuse for further participation any time during the study.

MRI and Pharmacokinetic Analysis

DCE-MRI was performed using a 1.5-Tesla system (Siemens, Erlangen, Germany) with a T1-weighted two-dimensional gradient echo sequence (repetition time 7.0 ms, echo time 3.9 ms, matrix 256 × 256, bandwidth: 260 Hz/sec, 15 axial slices, 22 sequential repetitions). Gadolinium-DTPA (Magnevist, Schering AG, Germany) was administrated by slow injection rate (0.1 mmol/kg body weight within 30 seconds) after three non–contrast-enhanced images using a power injector (Tomojet-System, GE Healthcare, Buckinghamshire, UK). Scans were obtained during continuous shallow breathing.

DCE-MRI source data were postprocessed using an in-house–developed pharmacokinetic two-compartment model (14,15). Based on this color-coded map, signal intensity time curves were calculated for individual region of interest (ROI) (total tumor and hot spot [ie, an area with high vascularity presenting with bright colors on the parameter images] and normal tissues [muscle, liver and spleen parenchyma]). Effort was made to retrospectively depict the sampling areas from the biopsy spot. Three pharmacokinetic parameters, amplitude (Amp), redistribution rate constant (k_{ep}), and elimination rate constant (k_{el}) were calculated for each ROI.

Microvessel Density: CD-34 Staining of MM

The microvessel density has been defined by absolute numbers of the so-called diffusion length, which is the mean distance of tumor cells from their nearest vessel (main supplying vessel, in micrometers) and the absolute size of the vessels measured in area (square micrometers) and diameter (micrometers) (diffusion length: mean distance of tumor cells to the nearest vessel detected by CD-34 immune stain). Paraffin blocks of formalin-fixed wedge biopsies were obtained by transthoracic biopsy procedure from representative tumor areas, which were defined by morphologic MRI. Biopsies were cut into 3–5 μm thick glass slide sections, deparaffinized, rehydrated, and then subjected to conventional peroxidase anti-peroxidase method (PAP) immunohistochemistry (2,16). The histologic diagnosis of MM and the exclusion of metastatic carcinoma into the pleura were based on the light microscopic findings obtained with hematoxylin and eosin, periodic acid-Schiff stain (PAS) with and without diastase digestion, and Giemsa stains (2,16).