Technetium-99m-hexakis-2-methoxyisobutylisonitrile scintigraphy and multidrug resistance-related protein expression in human primary lung cancer

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Abstract
Objective The occurrence of multidrug resistance (MDR) is a major cause of resistance to chemotherapeutic agents in patients with lung cancer, in part owing to the overexpression of MDR-related proteins. Technetium-99m-hexakis-2-methoxyisobutylisonitrile (99mTc-MIBI) has been shown to be a substrate for some MDR-related proteins. The aim of this study is to evaluate the role of 99mTc-MIBI scintigraphy for functional imaging of MDR-related protein phenotypes.

Methods To determine the correlation between 99mTc-MIBI scintigraphy and the expression level of P-glycoprotein (Pgp), multidrug-resistance protein (MRP), and glutathione-S-transferase Pi (GSTπ), 26 patients (17 men and 9 women, median age 57.5 years) with primary lung cancer were investigated. Following intravenous administration of 925 MBq 99mTc-MIBI, single-photon emission computerized tomography (SPECT) and computed tomography (CT) were performed at 15 min and 2 h. On the basis of the fused images, tumor to background (T/B) ratio of both early and delayed images, and washout rate (WR%) of 99mTc-MIBI were calculated. The immunohistochemical staining of Pgp, MRP, and GSTπ was performed, and the expression level was semiquantititated using a pathoimage analysis system. The imaging results were compared with the status of Pgp, MRP, and GSTπ expression.

Results The WR% of 99mTc-MIBI showed a significant positive correlation with Pgp expression ($r = 0.560$, $P = 0.003$), as no correlation was observed between WR% and MRP or GSTπ ($r = 0.354$, $P = 0.076$; $r = 0.324$, $P = 0.106$). Neither early T/B nor delayed T/B correlated with the expression level of Pgp, MRP, and GSTπ. WR%, Pgp, and GSTπ expression showed significant differences between squamous cell carcinoma (group A) and adenocarcinoma (group B). There was no significant difference among Pgp, MRP, and GSTπ expression levels in any cases ($P > 0.05$).

Conclusions Our data confirmed that 99mTc-MIBI scintigraphy is useful for determining the MDR caused by Pgp in patients with primary lung cancer.

Keywords Technetium-99m-hexakis-2-methoxyisobutylisonitrile · Multidrug-resistance-related protein · Primary lung cancer · Multidrug resistance

Introduction
Chemotherapeutic agents are widely used in the treatment of lung cancer, but they are not always effective in all cases. The occurrence of multidrug resistance (MDR) is a factor in the failure of chemotherapy.

There are many mechanisms contributing to the occurrence of MDR. Recent studies have mainly focused on the expression of MDR-related proteins. Clinical studies have shown that overexpression of MDR-related proteins such as P-glycoprotein (Pgp), multidrug-resistance protein (MRP), and glutathione-S-transferase...
Pi (GST\(\pi\)) are prognostic indicators of a poor response to chemotherapy and poor prognosis of malignant tumors [1–6]. Traditional methods to detect the expression of MDR-related proteins include immunohistochemical staining, Northern/Western blot, flow cytometry, and polymerase chain reaction [7]. However, these methods do not yield information on dynamic function of MDR-related proteins in vivo.

Technetium-99m-hexakis-2-methoxyisobutylisonitrile (\(^{99m}\)Tc-MIBI) is a myocardial imaging agent, which is also useful for the detection of a variety of tumors. Research has confirmed \(^{99m}\)Tc-MIBI to be a transport substrate for both Pgp and MRP [8], which suggested that \(^{99m}\)Tc-MIBI scintigraphy could be suitable for clinical use as a functional and noninvasive method to monitor MDR-related proteins. Many researchers have reported that \(^{99m}\)Tc-MIBI imaging had a correlation with MDR-related proteins and could predict the response to chemotherapy in patients with malignant tumors [9–17]; however, some results are contradictory [18–21]. Extended studies in this field need to be conducted to validate existing data.

The aim of this study was to evaluate the role of \(^{99m}\)Tc-MIBI scintigraphy for functional imaging of MDR-related protein phenotypes in patients with primary lung cancer. By means of \(^{99m}\)Tc-MIBI scintigraphy and immunohistochemical study, we compared the uptake and washout of \(^{99m}\)Tc-MIBI with the expression level of Pgp, MRP, and GST\(\pi\) in patients with primary lung cancer.

Materials and methods

A total of 26 patients, 17 men and 9 women, aged between 25 years and 75 years (median age 57.5 years) were included in the study. All the patients were diagnosed with primary lung cancer, proved by histopathological examination of biopsy or surgery. Of the 26 patients, 16 had squamous cell carcinoma, 9 adenocarcinoma, and 1 small cell undifferentiated carcinoma.

\(^{99m}\)Tc-MIBI scintigraphy was performed in all the patients prior to starting any treatment, and all tumor specimens were obtained within 2 weeks of imaging for immunohistochemical analysis. The study protocol was approved by the local ethics committee, and all the patients gave an informed consent. Commercial \(^{99m}\)Tc-MIBI was obtained from Beijing Atom HighTech (Beijing, China). Radiochemical purity was always higher than 95%. Each patient received 925 MBq \(^{99m}\)Tc-MIBI by intravenous injection in the arm contralateral to the thoracic mass. Single-photon emission computed tomography (SPECT) was performed using a coincident dual-detector camera-based system with a low-output computed tomography (CT) device (GE Millium VG Hawkeye, General Electric Medical Systems, Milwaukee, WI, USA). Thoracic tomography in the coronal, sagittal, and transaxial planes was started 15 min (early) and 2 h (delayed) following injection of \(^{99m}\)Tc-MIBI, using a gamma camera equipped with a low-energy, general-purpose collimator. The energy peak was centered at 140 keV with a 10% window. The images were attenuation-corrected using X-ray and reconstructed with the iteration method. CT was performed immediately following ECT acquisition as the patient was lying in the same position. Fused CT plus \(^{99m}\)Tc-MIBI images were acquired using fusion software. \(^{99m}\)Tc-MIBI tomographic images corresponded to the CT slices.

To calculate tumor to background ratios (T/B), transverse fused images, which displayed the clearest lesion, were selected and manual region of interest (ROI) was set on the lesion (T) and symmetrical ROI on the contralateral side (B) on early and delayed images. The counts of ROI were recorded and T/B of both early and delayed images was calculated. The washout rate per hour (WR\%\) of \(^{99m}\)Tc-MIBI from the lesion was determined using the following formula:

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WR\% = \left(\frac{T/B\; 15\text{min} - (T/B)\; 2\text{h}}{(T/B)\; 15\text{min}}\right) \times 100.
\]

The immunohistochemical staining was performed according to the standard streptavidin–biotin method. The staining was done on the close section of tumors. The primary antibodies were the mouse monoclonal antibodies (MoAb) of Pgp, MRP, and GST\(\pi\) (code: ZM-0189, ZM-0345, ZM-0210, Beijing Zhongshan Golden Bridge Biotechnology, Beijing, China), which have a specific reaction with the corresponding human proteins. Sections 3\(\mu\)m thick were cut from 10% formalin-fixed and paraffin-embedded lung cancer tissue and mounted on slides coated with poly-L-lysine. The slides were washed with distilled water and placed in a lam carrier. The sections were treated with sodium citrate solution (pH 6.0) and heated in a microwave oven for 20 min. After washing with distilled water, the slides were treated for 3 min with Tris-buffer (pH 7.6) washing solution, and a protein-blocking procedure was applied to inhibit non-specific protein binding. The specimens were incubated with primary antibody diluted in 1:200 overnight at 4°C, then treated with the biotinylated anti-mouse/goat immunoglobulin and peroxidase-conjugated streptavidin. Labeling was developed in a 3,3-diaminobenzidine tetrahydrochloride (DAB) substrate kit (code: SP-9000, Zymed, San Francisco, CA, USA) and nuclei were counterstained with hematoxylin.