行政院國家科學委員會專題研究計畫成果報告

計畫名稱-中文： 使用鎵-99m MIBI惡性淋巴瘤造影結果與P-醣蛋白-多藥物抗藥性(Pgp-mdr)及多藥物抗藥性相關蛋白質(MRP)基因

計畫名稱-英文： The study of technetium-99m-sestamibi scan results in malignant lymphomas compared with p-glycoprotein expression, multidrug resistance related protein expression

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主持人： 高嘉鴻　**執行機關：**中國醫藥學院醫學系

一、中文摘要

本研究之目的在利用鎵-99m MIBI 造影來預估惡性淋巴瘤之化療結果，並與P醣蛋白（Pgp）及多藥物抗藥性相關蛋白（MRP）之表現及其他預後因子做比較。

25位惡性淋巴瘤病人於化療前進入本研究，於靜脈注射鎵-99m MIBI 10分鐘後進行造影並判讀造影之結果及計算腫瘤背景之比值（T/B ratio)。所有25個惡性淋巴瘤加以切片進行免疫組織化及分析（IHA)以測定Pgp及MRP的表現。並在化療結束後第1-2年進行化療反應的評估。結果顯示15位化療反應良好的病人有明顯比較高的T/B ratio（3.3±0.6對1.2±0.1)。所有15位化療反應良好的病人有陽性的鎵-99m MIBI造影結果及陰性的Pgp及MRP表現。所有10位化療反應不良的病人有陰性的鎵-99m MIBI造影結果及陽性的Pgp及MRP表現。但其他的預後因子對於化療反應並無相關性。因此本研究結果顯示鎵-99m MIBI造影可以呈現Pgp及MRP表現且比較其它預後因子更能正確預測惡性淋巴瘤化療的結果。

關鍵詞： 惡性淋巴瘤、鎵-99m MIBI 造影、化療、P-醣蛋白表現、多藥物抗藥性相關蛋白表現

Abstract

The purpose of this study was to predict the response of malignant lymphomas (ML) to chemotherapy by technetium-99m methoxyisobutylisonitrile (Tc-MIBI) scan and to compare it with the predictive ability of P-glycoprotein (Pgp) expression, multidrug resistance related protein (MRP) expression and other prognosis factors. Twenty-five ML patients were enrolled in this study prior to initiation of chemotherapy. Images were obtained 10 minutes after intravenous injection of Tc-MIBI, interpreted visually and the tumor-to-background (T/B) ratios calculated. Immunohistochemical analyses were performed on sections of the biopsy specimens to determine Pgp and MRP expression. Chemotherapy response was evaluated in the first 1-2 years after completion of chemotherapy. The mean T/B ratio of the 15 patients with good response (3.3 ± 0.6) was significantly higher than that of the 10 patients with poor response (1.2 ± 0.1). All 15 patients with good chemotherapy response had positive Tc-MIBI scan results and negative Pgp and MRP expression. All 10 patients with poor response had negative Tc-MIBI scan results and either positive Pgp or MRP expression. Other prognosis factors showed no significant difference in the incidence of good and poor responses. Tc-MIBI scan results represent Pgp or MRP expression better than other prognosis factors more accurately and predict chemotherapy response in ML patients.

Keywords: Malignant Lymphoma, Technetium-99m Methoxyisobutylisonitrile, Chemotherapy Response, P-glycoprotein Expression, Multidrug Resistance Related Protein Expression.

Background and Purpose

Chemotherapy is the primary therapeutic modality for many maliganat lymphoma (ML) including all non-Hodgkin's lymphoma (NHL) and many

cases of Hodgkin's disease (HD) [1-3]. Since resistance to chemotherapeutic agents is a major cause of treatment failure, the goal of chemotherapy for ML is to avoid possible resistance and to achieve the highest response.

The mechanism of tumor uptake of technetium-99m methoxyisobutylisonitrile (Tc-MIBI) may involve binding to the cytosol of the tumor cell [4]. The cationic charge and lipophilicity of Tc-MIBI, mitochondrial and plasma membrane potentials of tumor cells, and cellular mitochondrial content can all play a significant role in tumor uptake of this agent [5], or the uptake may be caused by indirect phenomena such as increased tumor blood flow and capillary permeability. Tc-MIBI scan has been used to successfully predict the chemotherapy response of ML [6,7]. However, no studies have compared the relationship between Tc-MIBI scan results and Pgp or MRP expression in predicting chemotherapy response of ML. Therefore, the aim of this study was to compare Tc-MIBI scan results, immunohistochemical analyses of Pgp and MRP expression, and other prognosis factors as predictors of chemotherapy response in ML patients.

PATIENTS AND METHODS

Patients. Twenty-five patients (13 men, 12 women; age range 25-65 years; mean age: 46.2±12.3 years) with ML (11 with HD and 14 with NHL) were included in the study and underwent Tc-99m MIBI scans prior to chemotherapy (Table I). The classification of ML used followed the Lukes-and-Butler and updated Kiel systems [8]. After Tc-MIBI scans, the 11 HD patients received chemotherapy regimens with nitrogen mustard (mechlorethamine), vincristine, procarbazine, and prednisone (MOPP) alternating with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD); and the 14 NHL patients received chemotherapy regimens with cyclophosphamide, doxorubicin, vincristine, and predinisone (CHOP) protocols [1-3].

Technetium-99m Methoxyisobutylisonitrile Scan. The imaging procedure began 30 min after oral intake of 500mg perchlorate prevent abnormal uptake of free Tc-99m pertechnetate. A commercial MIBI preparation (max. 5.56Gb (150mCi) in approximately 1 to 3ml) was obtained from The Du Pont Merck Pharmaceutical Co. (Cardiolite, Billerica, MA, USA). The labeling and quality control procedures were carried out according to the manufacturer's instructions. Labeling efficiencies were all higher than 95 percent. Each patient was positioned supine on the imaging table with the chest strapped to prevent motion. Because of physiological Tc-MIBI accumulation in abdominal and pelvic organs, visualization of ML located in abdominal and pelvic regions is unreliable. In this study, images of supradiaphragmatic ML were obtained 10 minutes after intravenous injection of 740MBq (20mCi) Tc-MIBI in the anterior and posterior projection. The equipment consisted of a large field-of-view gamma camera fitted with a low-energy, high-resolution collimator. A single 20% energy window was set at 140 keV, and 500K counts were obtained for each static image. Tumor-to-background (T/B) ratios were calculated as the mean counts over the region of interest (ROI) of the tumor outlined in the largest lesion ÷ the mean counts over the ROI of background defined as the contralateral normal side for the neck and axilia lesions or normal soft-tissue of the thorax for mediastinal lesions. Tc-MIBI uptake in the lesions ≧ axillary soft-tissue background based on visual interpretation of at least 2 experienced nuclear medicine physicians was considered a positive Tc-MIBI scan results (Figs 1 and 2).

Immunohistochemical Staining. Formalin-fixed paraffin sections (5-ì m) were deparaffinized in an oven at 50℃ for 40 minutes, then hydrated with varying

concentrations of ethanol-water dilutions. For MRP immunohistochemical staining, antigen retrieval was performed by treatment in citrate buffer in a 700 W microwave oven for 5 minutes. Endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 minutes, followed by 5 minutes in phosphate buffer saline (PBS). The sections were incubated overnight in a moist chamber at 4 ℃ with primary antibody MRP QCRL-1 (10 μ g/ml, Signet Laboratories, Inc., Dedham, MA, USA) at 1:100 concentration. For Pgp immunohistochemical staining, endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 minutes. Antigen retrieval was performed by treatment with enzyme digestion in 0.1% trypsin in PBS for 5 minutes at room temperature and inhibited with 10% skim milk in PBS for 5 minutes. The sections were incubated for 2 hours in a moist chamber at 37℃ with primary antibody JSB-1 (50 μ g/ml, Boehringer Mannheim Biochemica, Germany) at 1:50 concentration. After three 5 minute washes in PBS buffer, detection of the primary antibody was performed with a link antibody according to the manufacturer's instructions (DAKO LSAB_ 2 System, Peroxidase, Dako Corporation, Carpinteria, CA, USA) [9-13]. Pgp and MRP expressions were interpreted by an experienced pathologist blind to clinical outcome as follows: negative = less than 10%, positive = 10% or more stained tumor cells (Figs 3 and 4).

Chemotherapy Response Evaluation. In this study, chemotherapy response of each patient was evaluated for the first 1-2 years after completion of treatment by clinical and radiological methods such as plain chest x-ray, chest computed tomography (CT) or magnetic resonance imaging (MRI), as well as head and neck CT or MRI, according to the following scale: 1. Complete response = no evidence of disease, 2. Partial response = at least 50% decrease in the sum of the products of the maximum perpendicular diameters of all measurable lesions, no

evidence of progression in any lesion and no new lesions, 3. No response = less than 25% increase in the sum of the products of the maximum perpendicular diameters of all measurable lesions, no evidence of progression in any lesion and no new lesions, and 4. Progressive disease = at least 25% increase in the sum of the products of the maximum perpendicular diameters of all measurable lesions and/or the appearance of new lesions. We defined complete and partial responses as good response, while no response and progressive disease were defined as poor response.

Statistical Analyses. T/B ratio was expressed as mean±standard deviation (SD). A Mann-Whitney U test was used to evaluate the difference in T/B ratios between patients with good versus poor response. The difference in incidence of good and poor response was evaluated for eight possible prognosis factors: positive versus negative Tc-MIBI scan results, positive versus negative Pgp expression, positive versus negative MRP expression, HD versus NHL, stage I-II versus stage III-IV, age > 40 years versus ≦40 years, and with versus without B symptoms (night sweats, fever > 38℃for 3 consecutive days, and unexplained weight loss of >10% body weight) [2,3]. A Chi-square test was used to determine if the frequency of good and poor response was the same for each pair. If the p value was < 0.05, the difference was considered significant.

Results

Detailed patient data are shown in Table I. The mean T/B ratio of the 15 patients with good response (3.3 ± 0.6) was significantly ($p < 0.01$) higher than that of the 10 patients with poor response (1.2 ± 0.1). All 15 (100%) patients with good response had positive Tc-MIBI scan results and negative Pgp and MRP expression. All 10 (100%) patients with poor response had negative Tc-MIBI scan results, among who 6 (60%) patients had positive Pgp expression while the other 4 (40%) had positive MRP

expression. Tc-MIBI scan results, Pgp expression, and MRP expression all showed significant differences in the rate of good and poor responses. However, no significant difference in the incidences of good and poor responses was found for lymphoma type, stage, age, or B symptoms (Table II).

Discussion

Our review of previous literature found only one paper which reported that 17 ML children with positive Tc-MIBI scan results and a higher mean T/B ratio had a better response to chemotherapy than 7 ML children with negative Tc-MIBI scan results and a lower mean T/B ratio [7]. Our results support their findings. However, their study did not examine the relation between other prognosis factors, Pgp or MRP expression and chemotherapy response.

The mechanism of chemotherapy resistance in ML is thought to involve expression of Pgp and MRP [11-14]. The retention of Tc-MIBI in tumor cells depends on Pgp and MRP expression, which function as ATP-dependent efflux pumps for many chemotherapy agents [15-18]. Therefore, in this study we used Tc-MIBI scan to predict the response of ML to chemotherapy. We found that positive Tc-MIBI scan results accurately predicted all good chemotherapy results, which were also related to negative Pgp and MRP expression. Moreover, negative Tc-MIBI scan results accurately predicted poor chemotherapy results in all patients with positive Pgp or MRP expression (Table I).

In our previous studies, only early chest images performed 10 minutes after intravenous injection of Tc-MIBI proved to be accurate enough to predict chemotherapy response in lung and breast cancer [17,19,20]. Therefore, in this study, we did not consider it necessary to do delayed chest imaging to calculate the tumor washout rate or retention index of Tc-MIBI to predict chemotherapy response. mRNA expression is not fully corrected with Pgp or

MRP expression in the tumor cell membrane, Tc-MIBI tumor uptake is directly based on the Pgp or MRP expression in the tumor cell membrane, and it was impossible to extract mRNA from the formalin-fixed paraffin sections of biopsy specimens [21-23]. Therefore, we directly detected Pgp or MRP expression by immunostaining to correct with Tc-MIBI tumor uptake (T/B ratio) in our study.

Based on our findings, we conclude Tc-MIBI scan results can represent Pgp and MRP expression for predicting chemotherapy response in ML patients. However, further studies including larger case numbers and for patients with chemotherapy relapses to repeat Tc-MIBI scan and recheck Pgp or MRP expression are necessary to confirm our findings.

計劃成果自評

本研究之成果能正確解釋鎵-99m MIBI造影正確預測惡性淋巴瘤化療反應結果的真正機轉，因此鎵-99m MIBI造影將可成為決定惡性淋巴瘤化療的結果是否有效的工具。

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