行政院國家科學委員會專題研究計畫　成果報告

動態對比劑顯影與顯微磁振造影影像在乳癌顯微循環及血管新生性之研究(2/2)

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計畫主持人：張允中

計畫參與人員：黃俊升，陳志宏，曾文毅，嚴逸芬

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動態對比劑顯影與顯微磁振造影影像

在乳癌顯微循環及血管新生性之研究（2/2）

Dynamic Contrast-enhanced and Microscopic MR imaging for Microcirculation and Angiogenesis of Breast Cancer （2/2）

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共同主持人：黃俊升
曾文毅
陳志宏
嚴逸芬
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(二)中、英文摘要及關鍵詞(keywords)。

Abstract

During the second-year project, magnetic resonance (MR) angiogenetic parameters derived from dynamic contrast enhanced (DCE) MRI was applied to the whole tumor.

We evaluated angiogenic compositions and tumor response in the course of neoadjuvant chemotherapy in patients with locally advanced breast cancer (LABC) using DCE MRI. Thirteen patients with LABC underwent serial DCE MRI (61 MR studies) during the course of chemotherapy. DCE MRI was quantified using a two-compartment model at pixel-by-pixel basis. Analysis of parametric histograms of amplitude, exchange rate k_{out} and peak enhancement over the whole tumor was performed. The distribution patterns of histograms were correlated with the tumor response. Initial kurtosis and standard deviation of amplitude before chemotherapy correlated with tumor response. Comparing the initial values with the values after the first course of chemotherapy, tumor response was associated with decrease in standard deviation of amplitude, and increase in kurtosis and decrease in standard deviation of k_{out}. Comparing the initial values with the values after completing the chemotherapy, tumors with better response were associated with increase in kurtosis (r^2=0.39), decrease in mean (r^2=0.71) and standard deviation (r^2=0.59) of amplitude, and decrease in mean of peak enhancement (r^2=0.50).

Our results suggested that tumors with better response tended to alter their internal compositions from heterogeneous to homogeneous distributions and to decrease in peak enhancement after chemotherapy. Serial analyses of parametric histograms of DCE MRI-derived angiogenic parameters can monitor the response of angiogenic compositions of a tumor throughout the course of chemotherapy, and is capable of predicting tumor response early in the course.
Keywords: magnetic resonance image, breast, chemotherapy

中文摘要

本研究第二年計劃，使用由磁振造影動態顯影衍生之血管新生性參數於整個乳房腫瘤之分析。我們評估局部侵襲性乳癌在化學治療當中以動態顯影磁振造影所獲相關血管新生性組成及腫瘤反應。在本研究中總共包含 13 個病患，接受了 61 次磁振造影檢查。所獲得之資料使用二隔間模式以像素為基礎加以分析。而整個腫瘤參數組織圖之分析包括輻度（amplitude）、交換速率（exchange rate, K_{out}）及最高顯影。腫瘤治療之反應與參數組織圖之分佈情況加以比較。我們發現初次檢查獲得輻度之 Kurtosis 及標準差與腫瘤反應相關，比較接受完第一次化學治療與初次化學治療前之參數值，我們發現腫瘤反應與輻度標準差下降，交換速率 K_{out} 的 Kurtosis 增加與 K_{out} 標準差之下降有關，而完成化學治療與初次化學治療前之比較，結果顯示輻度分佈之 Kurtosis 增加（r^2 = 0.39）、平均值下降（r^2 = 0.71）、標準差下降（r^2 = 0.59）及最高顯影之平均值下降（r^2 = 0.50）與腫瘤反應有關。

我們的研究結果顯示有較佳腫瘤治療反應者通常在治療中其內在組成會由較不均勻變為較均勻之分佈，而最高顯影之強度亦會在治療後下降，系列參數組織圖之分析可以監測在整個化學治療過程中血管新生性組成之變化，而且可以於治療早期預測腫瘤反應。
關鍵字：磁振造影、乳房、化學治療

(三)報告內容：請包括前言、研究目的、文獻探討、研究方法、結果與討論（含結論與建議）…等。

Materials and methods

1. *Patient recruitment*

(1) Thirteen patients (50.92 ±7.95 years of age) of LABC who fulfilled the criterion of tumor staging as T3 or T4 according to American Joint Committee on Cancer (AJCC) were enrolled in our study. None of these patients had distant metastasis before initiating neoadjuvant chemotherapy. All patients received at least 3 serial breast MR studies at the following time points: before receiving incision biopsy and initiating chemotherapy, following completion of the first course of chemotherapy, and before final surgical tumor resection (n= 8) or core biopsy (n= 5).

(2) All of the 13 cases received incision biopsy after completing the first MR study of breasts. Twelve cases were invasive ductal carcinoma and one case was invasive lobular carcinoma.

(3) The chemotherapy regimens included CEF (cyclophosphamide, epirubicin, fluorouracil) in 3 patients; palcitaxel plus cisplatin in 3 patients; weekly docetaxel plus oral UFUR (tegafur/uracil) in 6 patients, in which three of them received AC (doxorubicin, cyclophophamide) later due to unsatisfied response of docetaxel treatment; and vinorelbin plus weekly high dose 5-flurouracil/leucovorin 24 hours infusion in one patient.

(4) Patients were followed for 15±6.9 months (median 13 months) after completing their final MRI scan.

2. *MR technique and protocol of dynamic enhancement*

(1) Dynamic enhancement of MRI for bilateral breasts was performed in a 1.5T superconductive MR scanner (Sonata, Siemens, Erlangen, Germany) with dedicated breast coils. Patients were prone in position and received intravenous bolus injection of the contrast medium (Gadonilium-diethyltriaminepentaacetic acid, Gd-DTPA, with the dose of 2 mmole/kg) at the rate of 4 ml per second via automatic injector (Optistar MR injector, Mallinckrodt, MO, USA).

(2) A rapid scanning with 3D fast low angle shot (FLASH) pulse sequence coupled with elliptical scanning technique and partial Fourier acquisition were performed. Scan parameters were TE/TR = 3.1/12ms, flip angle =25 degrees, field of view = 110 mm × 320 to 360 mm, image matrix = 176×512, slice thickness= 4-5 mm, no gap, 30 slices, 14 seconds per acquisition, and total 46-50 acquisitions. The parameter setting resulted in a pixel resolution of approximately 625-700 μm. Both breasts were covered in dynamic study for the possibility of detecting multifocal or bilateral breast cancers in our patients. Bolus injection was started at the fourth acquisition in order to achieve steady state of magnetization and to avoid flow-related enhancement.

3. *Analysis of dynamic MR data*

(1) Time-intensity curves obtained from DCE MRI were analyzed at pixel-by-pixel basis with a two-compartmental model proposed by Brix et al. (Brix *et al* 1991). Two angiogenic parameters, namely, amplitude and redistribution rate constant (k_{out}), were determined by fitting the time-intensity curves (Buckley *et al* 1994). Amplitude represents initial slope of enhancement and

redistribution rate constant (k_{out}) represents the exchange rate of contrast agent between the intravascular and extravascular extracellular space (Buckley *et al* 1994, Knopp *et al* 1999). Color maps of amplitude and k_{out} were generated for all imaging slices. We defined the tumor margin by manually tracing the subtraction images of dynamic enhancement showing best margin of the tumor. Total tumor volume was determined by summing the traced tumor volumes over all slices. Tumor response, defined as the ratio of the tumor volume at the final MRI scan to the tumor volume at the first scan, was then computed. Data analysis was performed with Matlab 6.1 (MathWorks Inc., Natick, MA, USA) and Mathematica 4.1 (Wolfram Media, Inc., Cahmpaign, IL, USA).

(2) Histograms of DCE MRI-derived angiogenic parameters of the whole tumor, including amplitude, k_{out} and peak enhancement were also obtained. To describe distribution patterns of tumor compositions, we calculated statistical indices including mean, standard deviation, skewness and kurtosis from the histograms of each angiogenic parameters.

結果與討論

Results

(1) There were 61 MR studies in total with the minimum of 3 studies for each case. The interval between the first and final MR studies was $4.88^{\pm}2.87$ months.

(2) There were eight cases with tumor reduction more than 50% of initial tumor volume (responders) and five with tumor reduction less than 50% of initial tumor volume (nonresponders). The tumor size was $188.88^{\pm}140.54$ ml before chemotherapy and was $70.27^{\pm}73.90$ ml after completing the course of chemotherapy.

(3) Color maps of amplitude showed that better tumor response was marked by a change from heterogeneous to homogeneous tumor compositions and decrease in peak enhancement. In contrast, there was little perceptible change of internal tumor compositions in the non-responders. In the responders, the histograms of amplitude showed progressive decrease and left shift of the peak and narrowing of the base. Such change was not observed in the histograms of k_{out} or histograms of both amplitude and k_{out} in the nonresponders.

(4) As listed in Table 1, kurtosis and standard deviation of amplitude before chemotherapy correlated with tumor response, (r^2=0.40 and r^2=0.37, respectively). After the first course of chemotherapy the changes in standard deviation of amplitude, and in kurtosis and standard deviation of k_{out} correlated with tumor response (r^2=0.63, 0.33 and 0.33, respectively). In comparing the final preoperative MRI study with the initial study, better tumor response was indicated by significant change in amplitude, showing increase in kurtosis (r^2=0.39), decrease in mean (r^2=0.71) and decrease in standard deviation (r^2=0.59), and in peak enhancement showing decrease in mean (r^2=0.50).

Discussion

(1) In this study, we analyzed signal time curves at pixel by pixel basis over bilateral whole breasts. This approach allowed complete mapping of tumor angiogenic compositions and facilitated visual

perception of tumor response to chemotherapy. We further characterized tumor heterogeneity in terms of histograms of angiogenic parameters. Using this metric we found that tumor heterogeneity in LABC was associated with tumor response to neoadjuvant chemotherapy.

(2) Neoadjuvant chemotherapy of LABC can result in satisfactory local control, and even increased overall survival, especially in patients with a complete clinical or histopathological response (Eltahir *et al* 1998). Mammography has been widely accepted as an important tool in detecting breast cancer but play a limited role in assessing the response of breast cancer to neoadjuvant chemotherapy. Its efficacy is even inferior to physical palpation in patients with dense breasts (Vinnicombe *et al* 1996, Weatherall *et al* 2001). Although serial biopsy during neoadjuvant chemotherapy provides a window to observe tumor response over time, small amount of tissue specimen is subjected to sampling bias in tumors with heterogeneous internal compositions. Recent studies also showed that DCE MRI is potentially useful in assessing tumor response to neoadjuvant chemotherapy (Abraham *et al* 1996, Rieber *et al* (1997, 2000), Wasser *et al* 2003, Delille *et al* 2003). It has been demonstrated that kinetics of Gd-DTPA uptake is contributed from microvessel density (MVD), vessel size and permeability (Buada *et al* 1996, Carriero *et al* 2002, Hulka *et al* 1997). To better explain kinetics of Gd-DTPA uptake, a pharmacokinetic two-compartment model was proposed to characterize time intensity curves in terms of amplitude, reditribution rate constant (k_{out}) (Knopp *et al* 1999, Brix *et al* 1991, Buckley *et al* 1994). In this model, amplitude was related to MVD, and exchange rate k_{out} was related to vascular permeability that was proportional to vascular endothelial growth factor (VEGF) (Knopp *et al* 1999). Pixel-by-pixel analysis of Gd-DTPA uptake time curves not only improves the specificity of diagnosis, it can produce parametric images that provide information about tumor heterogeneity, permeability and vascularity (Mussurakis *et al* 1997). DCE MRI is a useful tool to monitor the response of tumor angiogenesis and tailor the choice of therapeutic regimens for patients with LABC.

(3) Most of the DCE MRI studies analyzed averaged signal time curves from a tumor region in a single slice. However, this approach cannot indicate the heterogeneity of tumor compositions that may be one of the key factors affecting tumor response to chemotherapy. In our study, we compared the final tumor response with the dynamics of angiogenic compositions during serial neoadjuvant chemotherapy. Better tumor response was associated with a larger change of tumor compositions from heterogeneous to homogeneous distributions, and larger decrement of peak enhancement over the whole tumor. Before chemotherapy, lower kurtosis and higher standard deviation of amplitude correlated with better tumor response (r^2=0.40 and r^2=0.37, respectively). After the first course of chemotherapy, better responders showed larger decrease of standard deviation of amplitude, larger increase of kurtosis and decrease of standard deviation of k_{out}. In the final MR study, better responders showed larger increase of kurtosis, decrease of mean and standard deviation of amplitude and decrease of mean of peak enhancement. Our results suggest that the change in internal compositions may arise from different susceptibility of individual compositions to chemotherapy. Particularly, compositions with higher amplitude may be more susceptible to chemotherapy.

(4) A recent study by Wasser et al. revealed the potential capability of DCE MRI to assess therapeutic effects of neoadjuvant chemotherapy in breast cancer (Wasser *et al* 2003). Furthermore, a reduction of tumor size after chemotherapy was associated with a decrease of both k_{ep} (equivalent to k_{out}) (Padhani

and Husband 2001) and amplitude, where k_{ep} was a more sensitive indicator that began to drop right after the first cycle of chemotherapy. On the other hand, reduction of contrast enhancement was not a sign of tumor responsiveness (Wasser *et al* 2003). In our study, tumors with better response were more heterogeneous in the histograms of amplitude before chemotherapy (table 1). Histograms of k_{out} showed increased kurtosis and decreased standard deviation after the first course of chemotherapy. These findings suggest that compositions of tumor angiogenesis become more homogenous under effective chemotherapy. However, we did not find significant decrease in the mean values of k_{out} or amplitude. The disparity of this result from the previous study might be related to variable chemotherapy regimens employed in our study. Comparing with amplitude, we found that the response of k_{out} was rather inconsistent, suggesting that k_{out} might respond differently to different chemotherapy regimens.

(5) In conclusion, we demonstrated the usefulness of parametric histograms derived from DCE MRI to characterize heterogeneity of tumor compositions and to study the response of tumor angiogenesis to neoadjuvant chemotherapy. Using this method, in-vivo angiogenic compositions of the whole breast tumor mass can be monitored by serial MRI studies during the whole course of neoadjuvant chemotherapy. This method will be clinically useful in understanding pathophysiologic changes, predicting tumor response and guiding therapeutic approach.

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Table 1. Statistical analysis between different MRI-derived angiogenic parameters and tumor response at different time points

amplitude vs. tumor response												
	before C/T				after first C/T				after final C/T			
	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness
r^2	0.18	0.37*	0.40*	0.20	0.06	0.63*	0.17	0.21	0.71*	0.59*	0.39*	0.24
p	0.14	0.03*	0.02*	0.12	0.43	0.001*	0.16	0.11	0.0003*	0.002*	0.02*	0.09

k_{out} vs. tumor response												
	before C/T				after first C/T				after final C/T			
	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness
r^2	0.01	0.01	0.17	0.21	0.07	0.33*	0.33*	0.11	0.06	0.17	0.25	0.12
p	0.70	0.76	0.16	0.11	0.38	0.04*	0.04*	0.26	0.43	0.16	0.08	0.25

peak enhancement vs. tumor response												
	before C/T				after first C/T				after final C/T			
	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness	mean	SD	kurtosis	skewness
r^2	0.10	0.00	0.14	0.07	0.01	0.05	0.00	0.06	0.50*	0.14	0.29	0.04
p	0.28	0.93	0.22	0.38	0.74	0.44	0.84	0.40	0.01*	0.21	0.06	0.51

SD: standard deviation, C/T: chemotherapy, *$p < 0.05$.