Biochemical characterization of breast tumors by in vivo and in vitro magnetic resonance spectroscopy (MRS)

Uma Sharma · Naranamangalam R. Jagannathan

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Abstract Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have evolved as sensitive tools for anatomic and metabolic evaluation of breast cancer. In vivo MRS studies have documented the presence of choline containing compounds (tCho) as a reliable biochemical marker of malignancy and also useful for monitoring the tumor response to therapy. Recent studies on the absolute quantification of tCho are expected to provide cut-off values for discrimination of various breast pathologies. Addition of MRS investigation was also reported to increase the specificity of MRI. Further, ex vivo and in vitro MRS studies of intact tissues and tissue extracts provided several metabolites that were not be detected in vivo and provided insight into underlying biochemistry of the disease processes. In this review, we present briefly the role of various ^1H MRS methods used in breast cancer research and their potential in relation to diagnosis, monitoring of therapeutic response and metabolism.

Keywords Magnetic resonance spectroscopy (MRS) ·

In vivo · In vitro · Ex vivo · Breast cancer, Choline,

Biomarker · Therapeutic response

Introduction

Breast cancer is a major cause of mortality and morbidity in women worldwide (Greenlee et al. 2001). The early detection and treatment has significant impact on the quality of life and

overall survival of these patients. Traditional diagnostic methods like mammography and ultrasonography significantly increase the patient's survival prospects by early detection and therapy at an early stage. However, their specificity is low and also has limitations in the diagnosis of lesions in dense breast or microcalcification. Thus, there is a need for evaluation of additional techniques. Presently, both magnetic resonance imaging (MRI) and MR spectroscopy (MRS) have rapidly evolved as sensitive tools for anatomic and metabolic evaluation. MRI is useful for preoperative evaluation of patients planned for breast conserving surgery and for evaluation of intermediate mammographic findings including radiographically dense breasts (Kuhl 2007). It helps to avoid unnecessary biopsies for suspicious lesions seen on mammograms and also reported as a screening technique in high risk women. The sensitivity and specificity of MRI for breast cancer detection increased significantly with the use of contrast media (Kuhl 2007). Even though MRI provides information on the tumor extent and pathology, it does not provide information on the underlying biochemical processes associated with tumor progression or regression. However, this can be achieved through in vivo MRS, which provides information on the alterations of metabolic pathways during the disease processes by detection and quantification of metabolites. The objective of this review is to briefly describe various methods of breast proton (^1H) MRS and its role in the diagnosis and in monitoring tumor response to therapy.

In vivo MRS of breast

The concentration of various metabolites is altered or changed when the tissue characteristics change from normal to malignant and thus understanding the metabolomics of

U. Sharma · N. R. Jagannathan (✉)
Department of NMR & MRI Facility,
All India Institute of Medical Sciences,
New Delhi 110029, India
e-mail: jagan1954@hotmail.com

malignancy has become an important area of research. These metabolite changes provide an insight into the altered metabolic processes in diseased tissues and are useful in characterizing normal, benign, and malignant tissues. Further, the biochemical or metabolic information from a well-defined region of interest or volume element (voxel) is possible from in vivo MRS. Most in vivo breast ^1H MRS studies reported in the literature were carried out at 1.5 T. However, recently, there have been reports on breast MRS at 3 T (Do et al. 2006), 4 T (Bolan et al. 2003, 2004; Meisamy et al. 2004, 2005), and at 7 T (Bolan et al. 2006). Normally, a bilateral surface receive breast coil is used for optimal signal reception with the body coil as transmitter. Both single-voxel spectroscopy (SVS) and multi-voxel MRS [referred as chemical-shift imaging (CSI) or MR spectroscopic imaging (MRSI)] were used in the study of breast cancer patients. With MRSI, it is possible to assess simultaneously multiple lesions, distinguish lesion borders as well as the normal tissue.

Diagnosis of normal, benign, and malignant breast lesions

Sijens et al. (1988) using a surface coil reported the first breast MRS and showed high W - F ratio for malignant tissues compared to normal breast tissues of volunteers. The W - F ratio is determined from ^1H MR spectrum without water suppression. Normal breast tissue was dominated by fat (see Fig. 1a and b) while the tumor tissue showed higher water content (see Fig. 1c and d) (Jagannathan et al. 1998; Kumar et al. 2006). A change in lipid content was reported to be associated with tumor development and progression. Thomas et al. (2005) calculated the W - F ratio using cross peak volumes from localized two-dimensional correlated spectroscopy. Substantial overlap in W - F ratio of benign and malignant tissues limited its diagnostic utility (Jagannathan et al. 1998, 1999; Kumar et al. 2006).

In breast ^1H MRS, the huge signal from water hamper the detection of metabolites with lower concentration which is suppressed by chemical-shift selective radiofrequency pulses. Further, the overlap of dominant lipid peaks with other metabolites is another major drawback. Recently, sequences that simultaneously suppress both water and lipid resonances were also developed (Bolan et al. 2003; Baik et al. 2006; Mescher et al. 1996). Figure 1e shows the spectrum obtained with the simultaneous suppression of both the water and lipid peaks from the voxel shown in Fig. 1c. The water suppressed spectrum of malignant breast lesions showed an intense peak at 3.22 ppm corresponding to several Cho containing compounds such as phosphocholine (3.21 ppm), glycerophosphocholine (3.28 ppm), and free choline (3.19 ppm) and designated as total choline (tCho) peak. However, the major component of enhanced tCho in malignant cells has been identified as phosphocholine from cell line studies (Katz-Brull et al. 2002a).

Several groups have documented that tCho is specific to malignancy and can be used to differentiate malignant from benign breast tissues (Roebuck et al. 1998; Kvistad et al. 1999; Jagannathan et al. 1998, 1999, 2001; Cecil et al. 2001; Yeung et al. 2001; Katz-Brull et al. 2002b; Sharma et al. 2006a,b; Gary et al. 2007). The enhanced tCho signal in tumors has been suggested to be due to increase in both the biosynthetic pathway governed by choline kinase and the catabolic pathways governed by specific phospholipase (Glunde et al. 2004). Katz-Brull et al. (2002a) reviewed previous studies and reported overall combined sensitivity and specificity of MRS as 83% and 85%, respectively. Recently, with the inclusion of more data, Bartella et al. (2007) reported the increase in mean sensitivity and specificity as 87% with a positive predictive value as 90%. Gary et al. (2003) documented relation between tCho and expression of HER2/Neu. Several groups documented observation of tCho in normal breast tissue of lactating women (Kvistad et al. 1999; Jagannathan et al. 2001; Stanwell et al. 2005) which has been reported to arise due to increase in glycerophosphocholine (Stanwell et al. 2005).

Since tCho signal was observed in some benign and normal breast tissues, there is a need for quantitative estimation of tCho. Two approaches are in use: (a) semi-quantitative way by measuring the signal-to-noise ratio (SNR) of tCho resonance (ChoSNR), and (b) determination of the absolute concentration. ChoSNR is measured using the peak intensity (height) of Cho signal and the noise intensity in an off-resonance region of the spectrum. Sardanelli et al. (2008) reported a threshold ChoSNR value ≥ 1.9 for diagnosis of malignancy with 90% sensitivity and 89% specificity, while 97% sensitivity was obtained on exclusion of lesions smaller than 1 cm. Bartella et al. (2006) documented ChoSNR value as ≥ 2 for malignancy. Baik et al. (2008) reported a mean ChoSNR of 5.9 ± 3.4 (range 2.1–17.5) for malignant and 2.8 ± 0.8 (range, 1.8–4.3) for the benign lesions using CSI. A cut-off ChoSNR value of >3.2 as malignant gave 81% sensitivity, 78% specificity and 81% accuracy.

The absolute concentration of tCho can also be determined using external and internal water referencing. The concentration of tCho in the malignant tumors was reported (Roebuck et al. 1998) in the range of 0.7–2.1 mM using external reference method while with the water peak as an internal reference a wide range of 0.76–21.2 mmol/kg was reported (Baik et al. 2006). This variation demands further quantitation studies to determine the cut-off value of concentration in normal benign and malignant tissues. Meisamy et al. (2005) using internal water referencing method determined concentration of tCho and documented that quantitation provides higher sensitivity, specificity, accuracy, and inter-observer agreement. The use of internal reference method overcomes some of the limitations of the

Fig. 1 **a** T2-weighted fat-saturated MR sagittal image of a normal volunteer showing the voxel position from which proton MR spectrum **b** was obtained with out water or lipid suppression. **c** T2-weighted fat-saturated MR sagittal image of a patient suffering from infiltrating ductal carcinoma of breast showing the voxel from which the proton MR spectrum shown in **d** was obtained with out water or lipid suppression. The spectrum in **e** shows the tCho peak obtained with simultaneous water and lipid suppression from the same voxel as shown in **c**

external reference method like the need for correction for partial volume effect and separate calibration experiments. It also increases the specificity of MRS.

Role of in vivo MRS in the evaluation of therapeutic response

The use of W - F ratio to monitor the effect of neoadjuvant chemotherapy (NACT) on breast tumors was reported (Jagannathan et al. 1998; Kumar et al. 2006). The reduction of W - F ratio following NACT in association

with the reduction of the primary tumor size indicates its use as a noninvasive indicator of favorable clinical outcome of therapy. Reduction of tCho as a marker to monitor treatment response was also reported (Jagannathan et al. 2001). Meisamy et al. (2004) reported a change in tCho concentration within 24 h of chemotherapy. Recently, we reported preliminary findings of the potential of ChoSNR, tumor volume and diameter in the assessment of tumor response of breast cancer patients undergoing NACT using sequential MRSI and conventional MRI (Danishad et al. 2007).

Ex vivo and in vitro proton MRS studies

Ex vivo and in vitro ^1H MRS of tissue samples or fine needle aspirates (FNA) offer comprehensive biochemical characterization with better sensitivity and resolution as they can be performed at high-fields allowing observation of a large number of metabolites than that could not be observed in vivo. Moreover, the use of multivariate analysis of the in vitro or ex vivo MRS data has been shown to be useful for classifying various breast pathologies. Recently, Whitehead and Kieber-Emmons (2005) reviewed the metabonomic studies carried out using in vitro and ex vivo MRS in breast cancer.

High-resolution magic angle spinning (HR MAS) NMR

Highly-resolved spectra of intact breast cancer specimens were reported using HR MAS ^1H MRS (Cheng et al. 1998; Sitter et al. 2002). They reported that metabolic markers like phosphocholine, lactate, and lipids correlate with the histopathological grade and improved the accuracy of the diagnosis. Sitter et al. (2002) compared HR ^1H MAS of breast cancer tissues with conventional high-resolution spectra of perchloric acid extracts of the same tissue type. Spectral resolution of HR MAS was reported to be comparable to the spectra of extracts, and more than 30 different metabolites were assigned using 2D technique. The spectra of intact breast cancer tissue differ from perchloric acid extracts by the presence of lipids and fewer signals in the low field region. Comparison of HR ^1H MAS of breast cancer tissues with non-involved adjacent tissue from patients was also reported (Sitter et al. 2006). Multivariate models relating spectral data to histological grade, lymphatic spread, and hormone status were developed and it was shown that MR-determined metabolic phenotype may have a future role as a supplement for making decisions for adjuvant chemotherapy (Bathen et al. 2007).

Ex vivo MRS of FNA samples

The literature on high-resolution ^1H MR studies of FNA samples of the breast cancer specimen is sparse. Mackinnon et al. (1997) reported the use of choline to creatine ratio and pattern-recognition method in discriminating malignant from benign FNA samples with a sensitivity and specificity of 95% and 96%, respectively. Mountford et al. (2001) reported that using multivariate analysis of the MR spectra, malignant lesions could be discriminated from benign lesions with 98% sensitivity, 94% specificity and positive lymph nodes could be predicted (96% sensitivity, 94% specificity) in samples with low-fat content. Kumar et al. (2005) reported that in addition to the higher concentration of tCho, several other metabolites showed an increase in

malignant compared to benign aspirates. Spectra of different other cytopathologies also exhibited significant metabolic differences (Kumar et al. 2005).

In vitro MRS of breast tissues

As discussed earlier, in vitro ^1H MRS investigations have several advantages compared to in vivo. Spectra can be acquired at very high magnetic fields with increased sensitivity and spectral resolution facilitating identification of large number of metabolites. Determination of the absolute quantification of metabolites is also easy. Gribbestad et al. (1994) reported the in vitro ^1H MRS of normal and malignant breast tissues. They observed 30 metabolites with significant differences in the level of several metabolites between involved and non-involved breast tissues. A study carried out in our laboratory showed that tumor breast tissues have significantly higher concentration of lactate, alanine, lysine, acetate, glutamic acid, glutamine, phosphocreatine+ creatine, Cho, glycerophosphocholine (GPC)+phosphocholine (PC), myo-inositol in comparison to the non-involved tissues (Kumar et al. 2005). No significant difference was observed in the concentration of tyrosine, phenylalanine, glucose, and formate between invasive tumor and non-involved tissues.

Recently, Beckonert et al. (2003) reported that in vitro ^1H MRS of tissue extracts can be combined with appropriate pattern-recognition and visualization methods in order to monitor metabolic differences between various tissue types. Their results showed an increase of uridine diphosphate-hexose, phosphocholine, and phosphoethanolamine concentrations according to the tumor grade. A higher concentration of taurine was detected in the malignant samples. Myo-inositol and glucose content were elevated in control samples compared with malignant tissues. Both compounds also characterized different subgroups in the pool of unaffected tissue samples depending upon fat content or fibrosis. Lipid metabolites were elevated with high malignancy.

In vitro MRS of axillary nodes of breast cancer patients

The usefulness of in vitro ^1H MRS in detecting the metastases in axillary lymph nodes which help in avoiding node dissection in node negative patients was also reported. The biochemical changes associated with breast cancer metastases in axillary nodes and its use in the detection of axillary metastases in a clinical setting in comparison with conventional histopathology was reported by our group (Sharma et al. 2004; Seenu et al. 2005). Eighty-eight lymph nodes obtained from thirty patients with breast cancer were investigated. Forty metabolites were identified and the levels of GPC+PC, choline, lactate, alanine, and uridine-di-

phosphoglucose were elevated significantly in nodes with metastases. In addition, the intensity ratio of [(GPC+PC)/threonine] was higher in nodes with metastases and using this as marker, MRS detected the axillary metastases with a sensitivity, specificity and accuracy of 80%, 91%, and 88%, respectively. NACT lowered the concentrations of GPC+PC and [(GPC+PC)/threonine] ratio. The accuracy of MRS in detecting metastases was 75% in patients who received NACT as compared to 96% in those who did not. Our results demonstrated the potential of in vitro MRS in characterizing the axillary nodes metastases with reasonable accuracy and suggested that it can be complementary to histopathological evaluation and immunohistochemistry.

Conclusion

Presently, the specificity of MRS was reported to be around 88%, however, the poor sensitivity and requirement of slightly larger lesion to detect a tCho signal is one of the limitations. Several centers have begun to complement breast MRI studies with MRS to gain the advantage of high specificity. However, recent advances of in vivo MRS quantitative methods, high-field MR systems, and improved design of breast coils with high sensitivity are expected to provide answers to various vital clinical questions related to early diagnosis, detection of small lesions, increased specificity and early prediction of tumor response. Further, in vitro and ex vivo ^1H MRS studies provide comprehensive biochemical characterization of intact breast cancer tissues or extracts.

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