In vivo and ex vivo choline metabolite profiles as biomarkers for treatment response in locally advanced human breast cancer

M. G. Heldahl[1], B. Sitter[1], T. F. Bathen[1], M. D. Cao[1], S. Lundgren[1,2], and I. S. Gribbestad[1]

[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Oncology, St. Olavs University Hospital, Trondheim, Norway

Background

Breast cancer patients show a highly varying response to neoadjuvant chemotherapy. New biomarkers are needed to obtain more individualized patient treatment regimes. Breast cancer demonstrates elevated levels of total choline compounds (tCho) in both ex vivo (1) and in vivo MR spectra (2). Also, the relative composition of the different choline compounds glycerophosphocholine (GPC), phosphocholine (PC) and choline (Cho) change with treatment in human breast cancer cell lines and xenografts (3, 4). The objective of this study was to investigate the effect of neoadjuvant chemotherapy on the level and composition of choline compounds in human breast cancer using ex vivo HR MAS and in vivo MRS.

Experimental

Tissue specimens from breast cancer patients were immediately frozen in liquid nitrogen after surgical dissection (n=16) or from core-needle biopsies (n=11). HR MAS MR spectroscopy was performed on a Bruker Avance DRX600 spectrometer according to previous described procedures (1). Standard pulse-acquired spectra with ERETIC quantification and spin-echo spectra (total echo time=285 ms) were obtained. Spectra were analyzed by curve fitting (Peak Fit, Seasolve). In vivo 1H MR spectra were obtained from breast cancer patients before (n=22) and during (n=15) neoadjuvant chemotherapy using a 3T Siemens Trio system (Siemens, Germany). Volume localization (size 10-15mm^3) was performed using the standard PRESS sequence (TE=135, TR=2000 ms and NS=128). Clinical patient response to treatment was determined according to UICC criteria (5).

Results and discussion

In vivo MRS: Contrast-enhanced MRI and in vivo MRS of a patient with locally advanced ductal carcinoma obtained before and three times during treatment is shown in Figure 1a. The tCho signal was detected in 15 of the 22 (68 %) patients with in vivo MRS. This is in accordance with previous reports (6, 7), and prove limitations in the sensitivity of in vivo breast MRS using present state-of-the-art technology. After one course of neoadjuvant chemotherapy, a tCho signal was detected in 6 of 12 patients (50 %). The six patients with no detectable level of tCho had clinical response of the treatment, while 4 responders and 2 non-responders were in the group of six patients with minor changes after one course of neoadjuvant chemotherapy. *Ex vivo MRS:* The HR MAS spectra of samples obtained from a patient group before neoadjuvant chemotherapy (n=8) and the same patients after treatment (n=8) showed a significant larger reduction in the tCho for the responder group (5.13 vs. 2.67 µmol/g, p=0.06) compared to non-responders (7.93 vs. 4.84 µmol/g tissue, p=0.16) during treatment.using paired T-test. The composition of choline metabolite composition is shown for responders (upper) and non-responders (lower) before (left) and after (right) treatment (Figure 1b). The non-responders showed a slightly higher GPC concentration (2.43±2.41 vs. 1.83±0.66µmol/g, not significant) compared to the responders in the pretreatment spectra. The PC level showed a trend to higher concentrations (not significant) in the spectra from the responders. After treatment, there were only minor differences between all the choline metabolites. Out of 11 patients with pretreatment core needle biopsies, six were defined as clinical responders and five as non-responders after neoadjuvant chemotherapy. The tCho concentration for the responders compared to non-responders was not significant different (3.05±3.97 vs. 1.76±2.96 µmol/g) and showed a large variation. The mean spectra from the two groups indicate differences in the composition of the metabolite profile, with a higher GPC level in the non-responders (Figure 1c). The HR MAS result demonstrates that detailed metabolic profiles of breast tissue specimens and needle biopsies can be studied before and during neoadjuvant chemotherapy. However, there is a large variation in the total choline and individual choline metabolites concentrations. More effort is needed to understand the tumor biology and microenvironmental influences on the metabolic profiles.

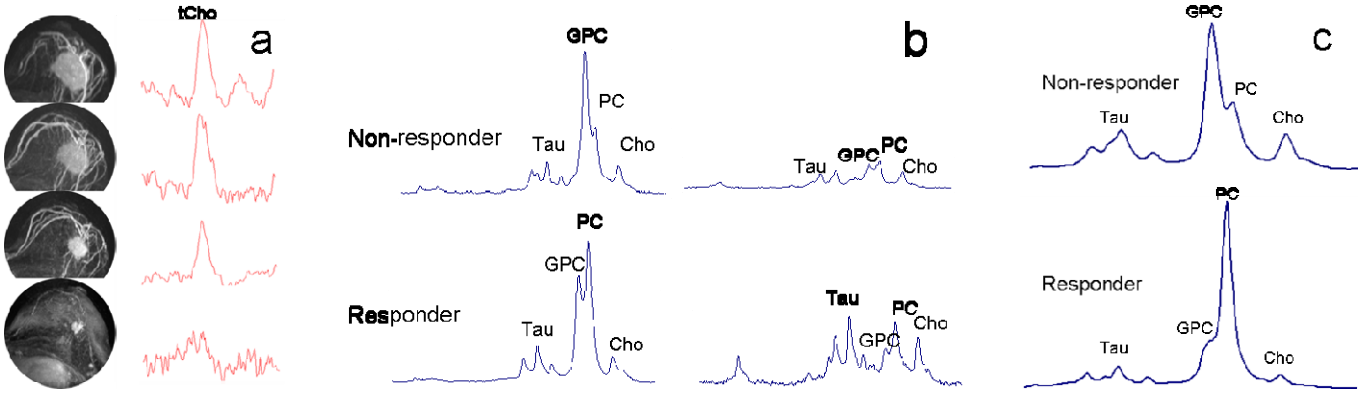

Figure 1: a) In vivo MRI and MRS. All images and spectra are from the same patient during the course of neoadjuvant chemotherapy (from the top: before treatment, after the 1st cycle of chemotherapy, after the 4th cycle of chemotherapy and before ablation). The patient is a non-responder at first. After a change in chemotherapy (after the 4th cycle) she becomes a responder. **b)** Tissue obtained from open surgical biopsy before therapy (to the left) and during main surgery (to the right), for a responder and a non-responder, examined with HR MAS MRS. **c)** Core-needle biopsies, before any treatment, examined with HR MAS MRS, with the mean spectrum for six clinical responders (upper), and mean spectrum for five clinical non-responders (lower).

Conclusion: The total choline level and choline metabolic profiles of human breast cancer change during neoadjuvant chemotherapy, and can be a useful biomarker for improved diagnosis and treatment monitoring. However, the underlying tumorbiological mechanisms are complex. HR MAS MR metabolic profiling of needle biopsies provide more detailed biochemical information compared to in vivo MRS. More knowledge about choline metabolism in human breast tumors are needed before the choline compounds can be used as a biomarker for treatment effect monitoring

References: 1. Sitter et al. NMR Biomed. 2006 ; 19 :30-40, 2. Gribbestad et al. JMRI. 1998 ; 8 : 1191-1197. 3. Degani et al. Int. J. Cancer 2006 ; 120. 4. Glunde et al. Pharmacogenomics 2006 ;7 (7) : 1109-1123. 5. Hayward et al. Br. J. Cancer 1977;35:292-298. 6. Bolan et al. Magn Res Med 2003; 50 :1134-1143. 7. Jagannathan et al. Br. J. Cancer 2001 ;84 (8), 1016-1022