Resistance and Sensitivity to Docetaxel treatment of Breast Cancer Tissue in Mice Assessed by Analysis of Choline Compounds with HRMAS NMR Spectroscopy

J. van Asten[1,2], T. F. Bathen[3], T. Buckle[4], C. Beekman[4], I. Gribbestad[3], F. van Leeuwen[4], and A. Heerschap[1]
[1]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [2]Biophysical Chemistry, Radboud University Nijmegen, Nijmegen, Netherlands, [3]Dept. of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, [4]Radiology and Nuclear Medicine, Division of Diagnostic Oncology, The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital

Introduction

Docetaxel is a chemotherapeutic drug that is commonly used to treat breast cancer [2]. However, resistance to chemotherapy is a common problem of cancer treatment [1]. Recently, two mouse strains were developed with breast tumors that are either resistant or sensitive to docetaxel [3]. With High Resolution Magic Angle Spinning (HRMAS) proton MR Spectroscopy (MRS) the levels of key compound tumor metabolism, such as those of choline can be assessed ex vivo. The **aim of this work** is to evaluate the value of tissue metabolite levels, assessed by HRMAS, to predict the effectiveness of docetaxel treatment using the sensitive/resistant mouse tumor models.

Methods

Mice, transplanted with either docetaxel resistant (n =11) or docetaxel sensitive (n =10) breast tumor tissue [3] were treated with docetaxel and the tumor response was monitored: 0 days (no treatment), 1 day, 3 days and 7 days after treatment. The treatment efficacy was studied with both volumetric tumor measurements (caliper measurements and computed tomography) and apoptosis (TUNEL) staining at imunnohistochemistry (IHC). Tumor sections from the different groups were subjected to HRMAS MRS (Bruker DRX 500MHz). The samples (4.5 - 11 mg), restricted to a 12ul sphere and mixed in D_2O, were measured at 4 ^{O}C and at a spinning rate of 4kHz, employing the CPMG sequence (T2filter/TR=30/5000ms)[4]. After Fourier Transform with 0.3 Hz exponential line broadening, the spectral range from 3.0 – 3.6 ppm was subjected to a Principal Component Analysis (PCA) to characterize the groups. In addition metabolite signals of glycerophosphocholine (GPC), phosphocholine (PC), choline (Cho), and creatine (Cr) were fitted with Lorentzian lines. Areas, normalized by Cr, were statistically analyzed by a two-tailed unpaired t-test. Correlation between metabolite ratios and treatment effect was assessed.

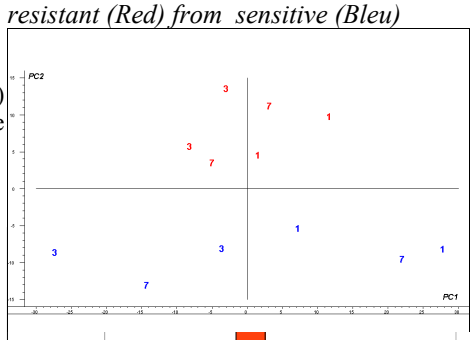
Results

Docetaxel sensitive and resistant tumor tissue during treatment were clearly separated by PCA (Fig. 1), which identified choline compounds as a major discriminating factor. Comparison of choline levels, demonstrated significantly higher total choline (tCho)/Cr and (GPC+PC)/Cr ratios for sensitive tumor tissue at day 0 (before) and day 1 after treatment (Fig. 2,3). With the volumetric measurements we observed a clear difference in response between the two tumor types; the sensitive group shows a clear chemo-response while the resistant does not . In addition, TUNEL IHC indicated that the level of apoptosis is increased at day 1 in the sensitive group. (Fig. 4).

Discussion

A difference in metabolite composition betweem the two tumor types was confirmed by the clear separation of their MRS spectra by PCA. Furthermore, increased choline compound levels were observed in the sensitive group before and one day after treatment coinciding with the highest apoptotic activity. These findings indicate that HRMAS MRS can be used for early response prediction.in docetaxel treatment.

Fig1 PCA analysis: PC2 separates resistant (Red) from sensitive (Bleu)



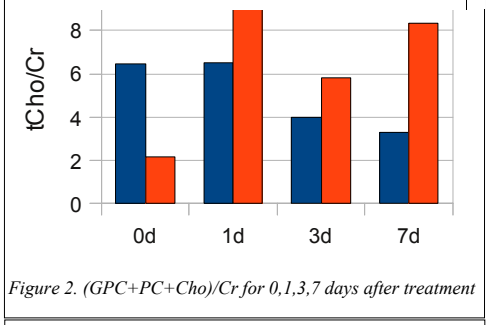


Figure 2. (GPC+PC+Cho)/Cr for 0,1,3,7 days after treatment

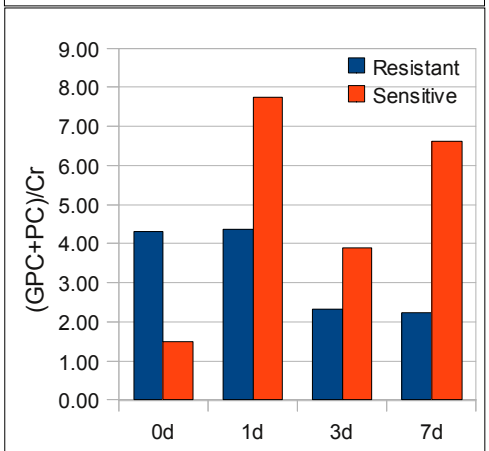


Figure 3:(GPC+PC)/Cr for 0,1,3,7 days after treatment

References
[1] S. De et al. Cancer Res. 2009 Oct 15;69(20):8035.
[2] H Joensuur et al Ann Oncol. 2009 Oct 9. [Epub ahead of print].
[3] S Rottenberg et al. PNAS, 2007, 104, 12117
[4] J van Asten et al. Magma 2008 21(6), 435.

Figure 4. TUNEL assay