Detection of lactate and other metabolites in ovarian tumours using ^1H NMR Spectroscopy

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INTRODUCTION

- Ovarian cancer is the 5th most frequent female cancer in UK (1)
- A non-invasive measurement of the metabolic profile of tumours is available with *in vivo* ^1H MR Spectroscopy (MRS) (2)
- Recent study suggested lactate as a prognostic parameter in tumour (3)
- However, the overlapping signals between lipid and lactate at 1.33 ppm is one of the challenges of metabolic observation in MRS
- An addition of inversion recovery pulse into the MR method can separate lipid and lactate because of the different T_1 value (4)

Figure 1. The Warburg Effect

AIMS

- To observe the metabolic profiles of ovarian tumours with single voxel (PRESS) and multivoxel (CSI) MR spectroscopy methods
- To find a robust method in observing metabolites and avoid the lipid contamination from the surrounding tissues and from within tumour cells

METHODS

1. Phantom Study

Two phantom models were made by a mixture of metabolites (Model 1: phosphocholine chloride, sodium lactate, alanine, and glycine; Model 2: alanine, sodium lactate, milk, and DMSO).

Study I: Analysis of the performances of two different coils (12 mm 2 turn solenoid coil and 39 mm quadrature coil)

Study II: An inversion recovery pulse was added in PRESS and CSI (T_1 = 10- 1000 ms)

Study III: Spin echo analysis (TE_1 = 8 ms – 768 ms)

2. In vivo analysis

The metabolic profiles of muscle and tumour tissues were compared using *in vivo* ^1H MRS. The A2780 Cell lines were injected into the rear dorsum of athymic CD1 nude mice and grown for 7-9 weeks to a size of 5-10 mm geometric mean diameter. The analysis in the animal model using PRESS and CSI were conducted with 299.64 Hz in 7T varian by using 12 mm solenoid coil.

3. Ex vivo analysis

Lactate assay of the biopsied tumour samples.

Figure 2. Illustration of the animal study

RESULTS

Phantom Study

Size: (2 mm) ³	Coil 12 mm	Coil 39 mm	Performance Ratio
Volume: 8 mm ³			(12 mm:: 39 mm)
TE_1 = 8 ms	151.4	53.6	2.8: 1
TE_1 = 128 ms	150.7	42.4	3.5: 1
Size: (3 mm) ³			
Volume: 27 mm ³			
TE_1 = 8 ms	498.2	105.5	4.7: 1
TE_1 = 128 ms	365.3	91.2	4.0: 1

Table 1. The Signal to Noise Ratio (SNR) from 12 mm coil and 39 mm coil

The value of the SNR is related to the echo time, voxel size, and coil size. Highest SNR was observed in 12 mm volume coil with TE_1 = 8 ms and 27 mm³ volume voxel.

Study I

Failed water suppression and loss of signal when increasing T_1 value.

Figure 3. The result of adding TI in PRESS

RESULTS

Study III

Figure 4A. Intensity of lactate (TE_1 = 8- 768 ms)

Figure 4B. Intensity of lactate (TE_1 = 128- 768 ms)

A new fitting line was formed for the lactate signal after the exclusion of TE_1 = 8 ms (figure 4B) because of lipid contamination at short echo time (TE_1 = 8 ms). The time constant is the value of the spin-spin relaxation time (T_2) (J = ~ 7.8 hz). The T_2 value changed from 142.42 (4A) to 255.59 (4B). ($rmse_A$ = 16.58 $rmse_B$ = 7.36)

Animal Study

Figure 5. MRS spectra of muscle and tumour tissues

Spectra obtained from muscle tissue in 8 mm³ voxel was compared to a spectra of tumour II in 27 mm³ voxel and tumour III in 8 mm³ voxel with TE_1 = 128 ms. The negative peak observed around 1.33 ppm in tumour III suggested the presence of lactate in addition to a positive lipid peak.

Figure 6. Colour map of CSI

Different colours represent the intensity of lipid peak in CSI. Red - blue = high - low intensity.

A same pattern of lactate intensity was observed in *in vivo* and *ex vivo* analysis (n = 3).

Figure 7. In vivo lactate analysis

Figure 8. Ex vivo lactate analysis

CONCLUSION

- ^1H MRS from muscle showed creatine and taurine signals. Tumours showed a choline signal and, in some cases, a signal which had phase properties consistent with lactate or alanine
- The choices of coil size, voxel size, and echo times are essential in MRS study
- Inversion recovery pulse is not necessary to use for metabolic observation
- Difficult analysis of CSI data due to inconsistent phasing and the number of voxels

FUTURE WORK

- A more robust MR method is needed. E.g. A comparison of the BASING pulse sequence, spectral/spatial water and lipid suppression pulses, and Outer Volume Suppression (OVS) as applied to tumour xenografts
- Bigger sample size
- Investigation of treatment responses using MRS
- Analysis of complete *ex vivo* measurement of the metabolites, histopathological observation, and Ca^{125} measurement of the tumor tissues
- A study of alanine as a tumour metabolite

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