

CHANGES IN LIPID DROPLET COMPOSITION DETECTED BY ^1H MRS DURING CISPLATIN TREATMENT OF DAOY CELLS

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INTRODUCTION

Several studies have reported a correlation between ^1H MRS visible lipids and successful cancer treatment indicating an imaging biomarker role for ^1H MRS detectable lipids in treatment monitoring. Cytoplasmic lipid droplets (LDs) are the main contributors to the ^1H MRS lipid signals and LDs are actively involved in cell death pathways. It has not been established whether the composition of lipids in the LDs alters when they accumulate during cell kill. In this study, human medulloblastoma cells were treated with cisplatin and accumulation of LDs was monitored by microscopy during cell kill. ^1H NMR was performed on whole cells, LDs isolated from homogenized cells and their extracts to investigate the ^1H MR spectral patterns of these accumulated lipids.

METHODS

Cell culture and chemotherapeutic treatment: DAOY cells were cultured in DMEM F:12 (GIBCO, Invitrogen Corporation, UK) supplemented with 10% (v/v) foetal calf serum (PAA, UK), 1% 200mM L-glutamine and 1% MEM non essential amino acid solution. 10 μM cisplatin (sigma Aldrich, UK) working solution, with the indicated concentration, was freshly made each time before use.

Nile red and DAPI staining: 4 $\mu\text{g/ml}$ Nile red and 0.4 $\mu\text{g/ml}$ DAPI was used to stain the cells after the cytospin. The slides were visualized using Nikon Eclipse E600 microscope using 100X objective lens and images were taken using a DXM1200 digital camera.

Isolation and Extraction: Sucrose gradient ultracentrifugation was used to isolate LDs from whole cell homogenate. Methanol and chloroform extraction was performed on isolated LDs and whole cells. Lipid extracts were re-suspended in 600 μl deuterated chloroform containing 0.03% (v/v) TSP (Sigma Aldrich, Dorset, UK).

^1H -NMR: HR-MAS NMR was performed on whole cell pellets on a Varian 600-MHz (14.1 T) vertical bore spectrometer using a 4-mm gHX nanoprobe (Varian NMR Inc) with a three channel Inova console running VNMRj software. A rotor speed of 2500 Hz was used for all experiments. ^1H NMR spectra of isolated LD and whole cell extracts were recorded on a Varian 600-MHz (14.1 T) vertical bore spectrometer using a HCN probe.

RESULTS

Lipid droplets accumulation with cell death: LDs appear as green vesicles in Nile red stained cells (Fig. 1), while cell nuclei were stained with DAPI and appear blue. There was an increase in the number of small LDs (diameter around 0.2 μm) by 12h of cisplatin treatment, while nuclei remained intact. A ring-like arrangement of small LDs was evident in cells when the LD size increased by 24h of cisplatin treatment. After 48h treatment, large LDs were seen with concomitant fragmentation of nuclei.

Spectra pattern of lipid alteration: ^1H HR-MAS NMR spectra of DAOY cells, as a function of cisplatin exposure times, are shown (Fig 2). The spectra were normalized to the macromolecule peak at 1.68ppm. An increase in peaks from unsaturated lipids (2.8 and 5.4ppm) is evident (Fig. 2). The ^1H NMR spectra from lipid extracts of isolated LDs are shown in Fig 3, which were normalized to the methyl group at 0.9ppm. The region around 5.4ppm, 3.4ppm and 1.3ppm as indicated with black rectangles in the control spectrum is expanded and plotted for all spectra. There is an increase in the lipid signal at 5.4ppm (CH=) and decrease in the 1.25ppm (-CH₂-CH₂-CH₂) and in the broader signal at 3.4ppm (phosphatidylcholine) (as pointed). No change was seen in the lipid signals at 5.4ppm and 1.25ppm in lipid extracts from whole cells (data not shown).

DISCUSSION

The increase in ^1H MRS detected lipids in cisplatin exposed DAOY cells was associated with the accumulation of LDs and occurred before DNA fragmentation. Isolation and extraction of LDs shows that there is an increase in unsaturated fatty acids and a decrease in saturated methylene groups in these LDs during drug treatment. A more detailed understanding of the changes in LD architecture can be obtained by isolating the LDs from cells furthering our understanding of the role of lipids in cell stress and death and identifying them as a potentially important imitable target for novel therapy.

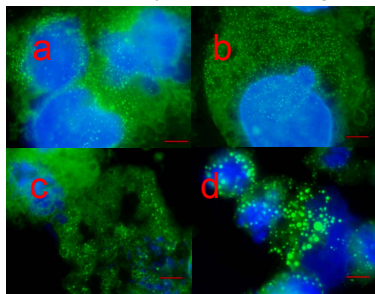


Fig 1 Nile red and DAPI staining of DAOY cells treated with 10 μM cisplatin after a control b 12h c 24h and d 48h. The size bar is 5 μm

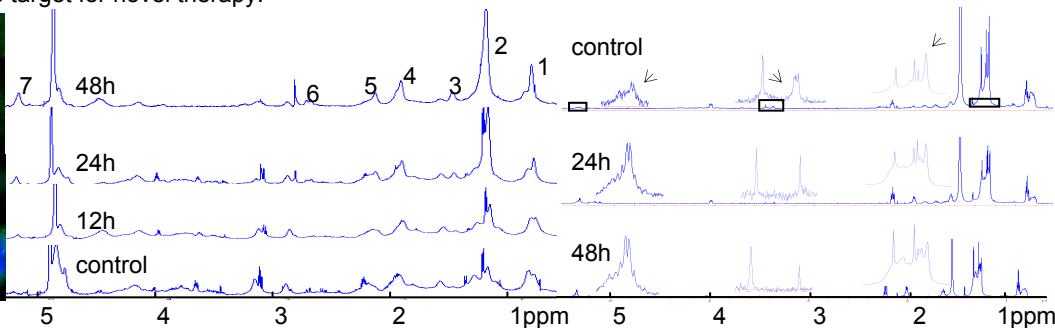


Fig 2 ^1H HR-MAS NMR spectra of DAOY cells treated with 10 μM cisplatin (Lipid signals labeled on the top spectrum are as follows: 1-CH₃, 2-CH₂-CH₂-CH₂, 3-CH₂-CH₂-C=O, 4-CH₂-CH₂-CH=, 5-CH₂-CH₂-C=O, 6-CH₂-CH₂-CH=, 7=CH)

Fig 3 ^1H NMR spectra of lipid extracts of isolated LDs from 10 μM cisplatin treated DAOY cells with expanded of region around 1.2, 3.4 and 5.4ppm