

UDP-GLCNAC AND UDP-GALNAC, AS DETECTED BY ¹H MRS, INCREASE IN THE EARLY PHASE OF CISPLATIN-INDUCED CELL DEATH IN BRAIN TUMOUR CELLS

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INTRODUCTION

Numerous ¹H MRS detectable metabolites have been shown to abruptly change in response to cell growth arrest or early events of cell death in cancer cells. However, little attention has been given to the metabolites in the low-field side of the ¹H MR spectrum. UDP-GlcNAc and UDP-GalNAc have key resonances in the low-field side of the ¹H MRS spectrum and are key precursors of cellular glycosylation, which is important to tumour progression. In this study, brain tumour cell lines with different sensitivities to cisplatin were examined by ¹H MRS. UDP-GlcNAc and UDPGalNAc were quantified together with indices of cell death.

METHODS

Cell line, chemotherapeutic treatment and detection of cell death: DAOY (human medulloblastoma), PFSK-1 (human PNET), BT4C (rat glioblastoma) and U87-MG (human glioblastoma) were used in this study. Cisplatin (sigma Aldrich, UK), at the indicated concentration, was freshly made each time before use. Alamar Blue assay (AbD Serotec, UK) was performed according to the standard protocol with three independent experiments performed for each cell line at each concentration. Each cell line was then exposed to 10 μ M cisplatin for up to 48 hours with DAPI staining and HR-MAS performed at 12, 24 and 48 hours. DAPI staining was performed using cytospin and fluorescence microscopy.

High-resolution magic angle spinning NMR (HR-MAS) : HR-MAS was performed 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. The pulse sequence consisted of a single 90° pulse, preceded by a 1s duration water presaturation pulse. The spectra were manually phased and referenced. After the baseline correction, they were normalized to the total intensity of the region from 1.4 to 4.5ppm. The signal intensity of the 7.85-8.05ppm and 5.2-5.4ppm regions was measured using integration to estimate the relative abundance of UDP-GlcNAc+UDP-GalNAc and unsaturated lipids, respectively.

RESULTS

Cell survival and cell death: The alamar blue assay (Fig 1) showed that the IC50 concentration of cisplatin for DAOY and BT4C cells was between 1 to 10 μ M while the value was 10-100 μ M for PFSK-1 and U87-MG cells. The DAPI staining (Fig 2) showed nuclear condensation or swelling after 48h exposure to 10 μ M-cisplatin in BT4C cells, while nuclei in U87-MG cells remained unchanged. The nuclear fragmentation was detected in DAOY cells using DAPI staining after 24h treatment and nuclei in PFSK-1 cells remained intact and normal with 48h treatment (data not shown).

Spectral patterns: The peaks at 7.98, 5.98 and 2.09ppm changed with cisplatin treatment in BT4C cells and were assigned to UDP-GlcNAc and UDP-GalNAc according to the literature. The assignment was confirmed by spiking cell extracts with these compounds (data not shown). The quantitative analysis of the 7.98ppm peaks and unsaturated lipid peaks at 5.3ppm (Fig 3) showed a large increase in glycosylated UDP compounds both in DAOY and BT4C cells with treatment. In responding cells, UDP-GlcNAc and UDP-GalNAc increased within 24 hours of exposure ($p < 0.05$, T-Test) before or at the onset of microscopic signs of initiation of cell death. ¹H MRS-lipids also increased substantially during cisplatin treatment in BT4C and DAOY cells (Fig. 3). U87-MG and PFSK-1 cells showed no change in glycosylated UDP compounds and only a modest increase in ¹H MRS detected lipids (Fig. 3)

DISCUSSION

At a 10 μ M concentration, cisplatin led to cell death in DAOY and BT4C cells by 48 hours exposure while U87-MG and PFSK-1 were resistant to the drug under these conditions. UDP-GlcNAc and UDP-GalNAc increased in responding cells (BT4C and DAOY) with a concomitant increase in ¹H MRS-lipids. Relatively strong lipid signals in U87-MG cells were due to necrosis associated with rapid growth rather than drug treatment, which is a similar feature observed in glioblastoma patients. In conclusion, UDP-GlcNAc and UDP-GalNAc are potential candidates for treatment response monitoring by ¹H MRS. The observation that these glycosylated UDP compounds increase in association with ¹H MRS lipids may provide mechanistic information about cell death processes.

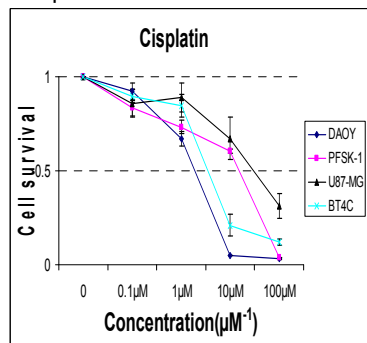


Fig 1 Alamar Blue cell survival curve.

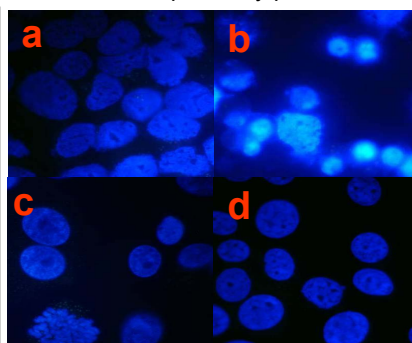


Fig 2 DAPI staining: a. BT4C control b. BT4C with 48h 10 μ M cisplatin c. U87-MG control d. U87-MG with 48h 10 μ M cisplatin

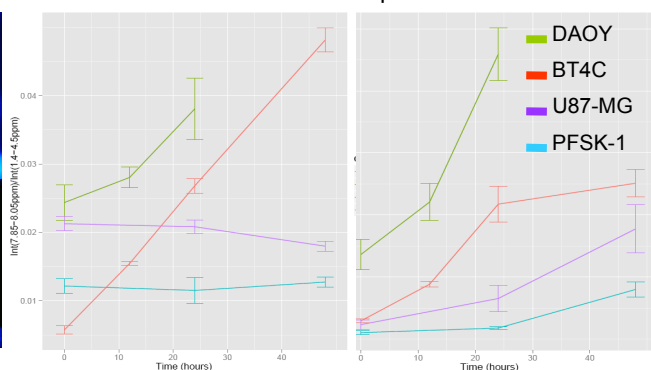


Fig 3 The quantitative analysis of the UDP-GlcNAc and UDP-GalNAc peak at 7.98ppm (left) and lipid peak at 5.3ppm (right) at 0h, 12h, 24h and 48h treatment using 10 μ M cisplatin.