MULTIPARAMETRIC NON INVASIVE MRS EVALUTATION OF CISPLATIN TREATMENT IN OVARIAN CANCER

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Introduction

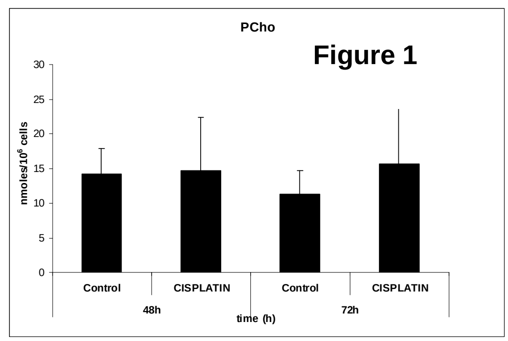
Epithelial ovarian cancer (EOC) is portrayed as an insidious disease or silent killer that causes no symptoms and generally cannot be recognized clinically until the disease formed widespread metastases within the abdominal cavity. Cisplatin in one of the most frequently used chemotherapeutics in the treatment of this tumor, but the clinical application of this drug in greatly limited by its toxicity. Evaluation and effectiveness of these anticancer therapies would be enhanced by noninvasive methods capable to quantitatively assess molecolar parameters of tumor responsiveness. MRS offers powerful approaches to detect metabolic alterations occurring in tumor cells following drug treatment and to investigate the biochemical pathways responsible for the observed spectral changes. Purpose of this study was to investigate the effects of cisplatin treatment on ^{1}H MRS signals of the human ovarian cancer cell line SKOV3ip, either cultured in vitro and or implanted in immunodeficient mice.

Methods

Cells: SKOV3ip was established from the in vivo passaged HER2-overexpressing SKOV3 cell line, according to the protocol by Yu et al.[Cancer Res 1993; 53: 891-8]. *Animal models*: EOC xenografts were obtained by subcutaneous implantation of 2 x 10^6 SKOV3ip cells in SCID mice. Three doses treatment (cisplatin 5 mg/kg, iv, weekly) started for a group of animals (CIS) when tumour reached a weight of about 80 mg (which corresponds to 8-15 days post injection); the control group (SAL) received only saline. Animal handling and treatment complied with European and Italian regulations. In vivo MRI-guided 1H-MRS analyses were conducted at 4.7 T on a Varian–Inova horizontal bore system. T2W MRI (TR/TE=3000/70ms) spin echo images with in plane resolution as high as 47 x 94 μm^2 and a thickness of 600 µm were acquired for tumor weight determination and for MRS voxel positioning. The diameter of s.c. tumors was measured weekly using a caliper. The weight was calculated according to the formula: weight (mg)= ½ x longest diameter (mm) x shorter diameter^2 (mm). Quantitative ^{1}H MRS analyses were performed by using a PRESS sequence (TR =4000ms, in order to minimise T1 relaxation losses and TE ranging from 23 to 256 ms) and assuming 80% of tumour water content. LCModel was used for the spectral fitting. High resolution MRS analyses were performed on intact cells and on cell extracts at 16.4 or 9.4 T (Bruker AVANCE). Statistical analyses were performed by T Student (significant differences, $P < 0.05$).

Results

The in vivo passaged SKOV3.ip cell variant exhibited an about two-fold higher intracellular phosphocholine (PCho) content compared with the parental SKOV3 cell line, associated with enhanced in vivo tumorigenicity (as detected by faster in vivo growth and reduced survival of the tumor-bearing mice). Cell exposure to cisplatin 5µM resulted in a substantial growth arrest (cell couting dropping to 40 ± 14.1 % (n=4) at 48h and 53.1±12.5 % at 72h (n=4) compared with untreated cells with no significant alteration of cell viability (>90%) measured by trypan blue exclusion test). A significant increase in narrow mobile lipid (ML) signals was detected in ^{1}H NMR spectra of intact cisplatin-treated cells compared with the control. MRS analysis of aqueous cell extracts (n=4) showed that the intracellular PCho levels was not significantly altered by cell exposure to cisplatin for 48 or 72h (Fig1).

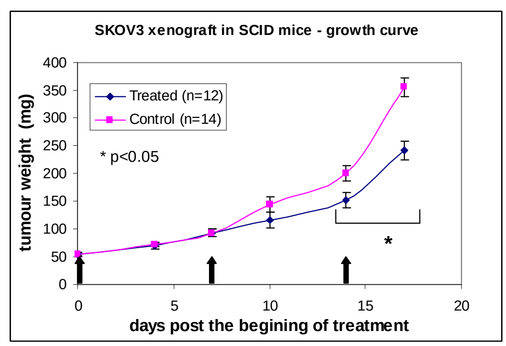


In vivo examinations showed a reduced tumour growth in the treated group as shown in Fig.2. The tCho peak increased during tumor growth in untreated animals (SAL); no significant differences were detected in tCho in cisplatin treated vs control animals (Table 1). Lipid signals also increased in xenografts during tumor growth but, because of their dependence on tumour size it was not possible to detect treatment-associated changes.

Table 1 – tCho (mM) in in vivo xenograft measured before and after 2 doses of treatment by ^{1}H MRS

	Pre	Post treat.
CIS (n=4)	3.7 ± 0.8	4.5 ± 0.6
SAL (n=4)	3.2 ± 0.6	4.7 ± 0.6

Figure 2



Conclusions

1) A higher basal level of intracellular PCho was associated in the cultured cells with the increased tumorogenity of SKOV3ip cells, compared with the wild-type SKOV3 cell line.
2) The increase in NMR-visible ML content, induced in SKOV3ip cells by in vitro exposure to cisplatin was found to correlate with drug response, indicating that ML signals could act as a potential pharmaco-dynamic biomarker of drug activity.
3) The PCho signal was not a direct index of cell proliferation, since there were no substantial differences in cisplatin-treated compared with control cells, either in vitro or in vivo (in spite of growth inhibition in both cases).

These results suggest that in these cells PCho acts as an indicator of tumor cell aggressiveness rather than as a direct end- point of cell proliferation. This finding points to the interest of further elucidating correlations between the PCho level and genomic expression of enzymes responsible for PCho accumulation (notably choline kinase and PC-specific phospholipases) for a better understanding of the role of this metabolite as diagnostic/prognostic index.

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