ESRF	**Experiment title:** CYTOTOXICITY OF CISPLATIN PLUS SYNCHROTRON RADIATION IN A549, IGROV-1 CANCER CELL LINES AND GLIOBLASTOMA CANCER STEM-LIKE CELLS	**Experiment number**: MD528
Beamline: ID17	**Date of experiment**: February 12, 2011 8h00 - February 13, 2011, 8h00	**Date of report**: April 7, 2011
Shifts: 3	Local contact(s)**: ALBERTO BRAVIN**	*Received at ESRF:*

Names and affiliations of applicants: Cecilia Ceresa[1], Gabriella Nicolini[1],Guido Cavaletti[1].

[1]Affiliation: Department of Neurosciences and Biomedical Technologies, University of Milan-Bicocca, Via Cadore, 48; Monza (MB), Italy

Report:

This study was aimed to investigate whether synchrotron radiation (SR) can enhance cisplatin (CDDP) cytotoxicity in two different human cancer cell lines of non-glial origin (A549 non small-cell lung cancer and IGROV-1 ovarian cancer cells) and in human glioblastoma cancer stem-like cells (CSCs).

Three different series of measurements have been performed within this proposal. This is the report for the third serie that took place on February 2011 as recuperation day for technical problems that occured on December 2010 not allowing the measurements on glioblastoma CSCs cells. In this experimental session the beamtime has been used in order to investigate whether synchrotron radiation (SR) can enhance cisplatin (CDDP) cytotoxicity in two different human glioblastoma CSCs: VIPI and DEMI cell lines.

Cell preparation

Glioblastoma CSCs were plated in (20000 cells/well) into flat bottom 96-well plates coated with laminin in DMEM/F-12 and Neurobasal (1:1) (Invitrogen), supplemented with B27 (Invitrogen), 10 ng/ml recombinant human bFGF (Milteniy) and 20 ng/ml recombinant human EGF (Milteniy), 2mM L-glutamine (Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen). After 24 hr both cell lines were treated with CDDP 3 μM for 24 hr. Untreated cells were used as control. After treatment plates were taken to the ID17 beamline to be irradiated and immediately washed with drug free medium.

Irradiation

Glioblastoma CSCs cells were irradiated with a total dose of 0, 1, 2, 4, 6 and 8 Gy. Cells were irradiated either above (78.8 KeV) and below (78.0 KeV) the Pt K absorption edge (platinum K-edge = 78.395) according to the following schedule:

DEMI and VIPI cells
1. Untreated control
2. CDDP 3 μM
4. SR irradiation dose 1Gy
5. CDDP 3μM +SR irradiation dose 1Gy
7. SR irradiation dose 2Gy
8. CDDP 3 μM +SR irradiation dose 2Gy
10. SR irradiation dose 4Gy
11. CDDP 3 μM +SR irradiation dose 4Gy
10. SR irradiation dose 6Gy
11. CDDP 3 μM +SR irradiation dose 6Gy
10. SR irradiation dose 8Gy
12. CDDP 3 μM +SR irradiation dose 8Gy

Cell survival determination

96 hr after irradiation cell survival was determined by Sulforhodamine-B (SRB) cell viability assay. At the end of the incubation period cells were fixed with 10% (wt/vol) trichloroacetic acid and stained for 15 minutes, after which the excess dye was removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader. The results obtained on DEMI glioblastoma CSCs and VIPI glioblastoma CSCs are reported in Fig.1 and Fig.2 respectively. Each experimental data point is represented as average value obteined from four replicates.

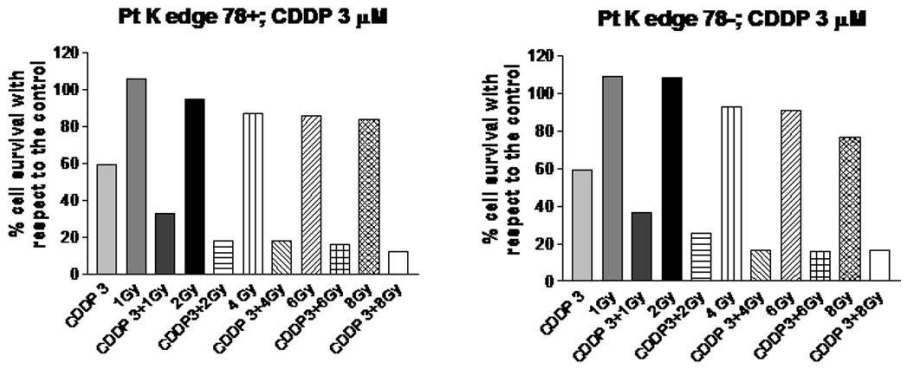

Fig.1 Effect of SR and CDDP combination in DEMI glioblastoma CSCs evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP 3 µM for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge)with a total dose of 1, 2, 4, 4, 6 or 8 Gy.

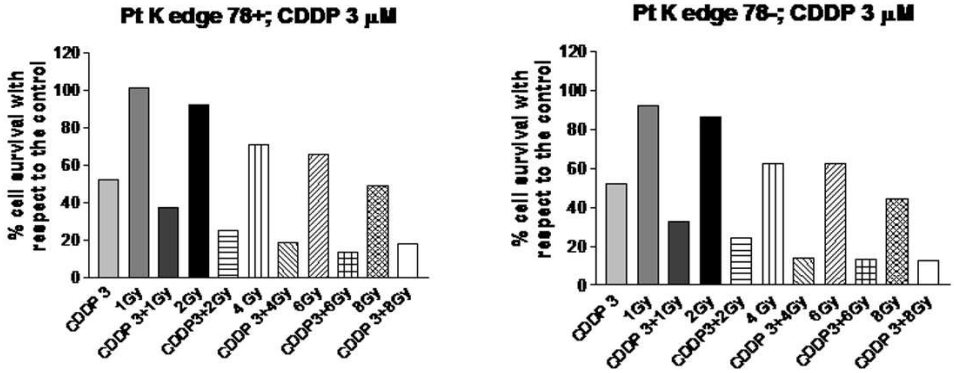

Fig.2 Effect of SR and CDDP combination in VIPI glioblastoma CSCs evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP 3 µM for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge)with a total dose of 1, 2, 4, 4, 6 or 8 Gy.

Discussion

Exposure to SR significantly enhances CDDP activity in both DEMI and VIPI human glioblastoma CSCs at 1, 2, 4, 6 and 8 Gy at both 78.8 keV (above Pt K-edge) and 78.0 KeV (below Pt K-edge) for both the CDDP concentrations used. Both cell lines, but in particular DEMI cells, showed a high resistance to SR. The CDDP dose used (3 µM) induced about 50% survival in both cell lines while a 10% was expected. In these conditions the SR effect is hidden by the CDDP cytotoxicity.

Further experiments would be usefull to clarify the CDDP toxicity in these cell lines in order to find a CDDP dose inducing between 5 and 10% of cel death.