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**: from: September 18, 2010 8h00 to: September 20, 2010 8h00	**Date of report**: April 7, 2011
Shifts:	Local contact(s)**: ALBERTO BRAVIN**	*Received at ESRF:*

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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 first serie that took place on September 2010. In this experimental session the beamtime has been used in order to confirm and finalize preliminary data about the effect of SR radiation in A549 and IGROV-1 cells previously obtained at beamline ID17 that have shown a SR significant enhancement of CDDP activity and survival in A549 and IGROV-1 cells.

Cell preparation
A549 and IGROV-1 cells were plated (1000 cells/well) into flat bottom 96-well plates in complete RPMI medium (Invitrogen) supplemented with 10% Foetal Bovine Serum (FBS) (Sigma), 2mM L-glutamine (Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen). After 24 hr cells were treated with CDDP for 24 hr. A549 cells were treated with CDDP 0.7 or 0.2 μM while IGROV-1 were treated with CDDP 0.2 or 0.05 μM. 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
A549 and IGROV-1 cells were irradiated with a total dose of 0, 1, 2, 4 and 6 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:

A549	IGROV-1
1. Untreated control	1. Untreated control
2. CDDP 0.7 μM	2. CDDP 0.2 μM
3. CDDP 0.2 μM	3. CDDP 0.05 μM
4. SR irradiation dose 1Gy	4. SR irradiation dose 1Gy
5. CDDP 0.7 μM +SR irradiation dose 1Gy	5. CDDP 0.2 μM +SR irradiation dose 1Gy
6. CDDP 0.2 μM +SR irradiation dose 1Gy	6. CDDP 0.05 μM +SR irradiation dose 1Gy
7. SR irradiation dose 2Gy	7. SR irradiation dose 2Gy
8. CDDP 0.7 μM +SR irradiation dose 2Gy	8. CDDP 0.2 μM +SR irradiation dose 2Gy
9. CDDP 0.2 μM +SR irradiation dose 2Gy	9. CDDP 0.05 μM +SR irradiation dose 2Gy
10. SR irradiation dose 4Gy	10. SR irradiation dose 4Gy
11. CDDP 0.7 μM +SR irradiation dose 4Gy	11. CDDP 0.2 μM +SR irradiation dose 4Gy
12. CDDP 0.2 μM +SR irradiation dose 4Gy	12. CDDP 0.05 μM +SR irradiation dose 4Gy
13. CDDP 0.7 μM +SR irradiation dose 6Gy	13. CDDP 0.2 μM +SR irradiation dose 6Gy
14. CDDP 0.2 μM +SR irradiation dose 6Gy	14. CDDP 0.05 μM +SR irradiation dose 6Gy

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 A549 and IGROV-1 cells are reported in Fig.1 and Fig.2 respectively. Each experimental data point is represented as average value obteined from four replicates.

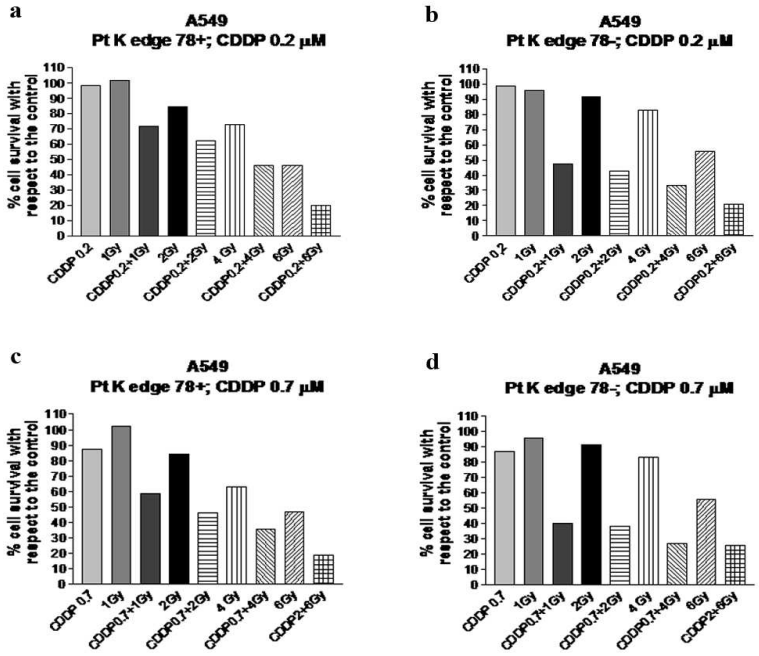

Fig.1 Effect of SR and CDDP combination in A549 cells evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP 0.2 or 0.7 μM for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge) with a total dose of 1, 2, 4 or 6 Gy. Irradiation with a total dose of 1, 2, 4 or 6 Gy significantly increased cell death with respect to CDDP alone and irradiation alone.

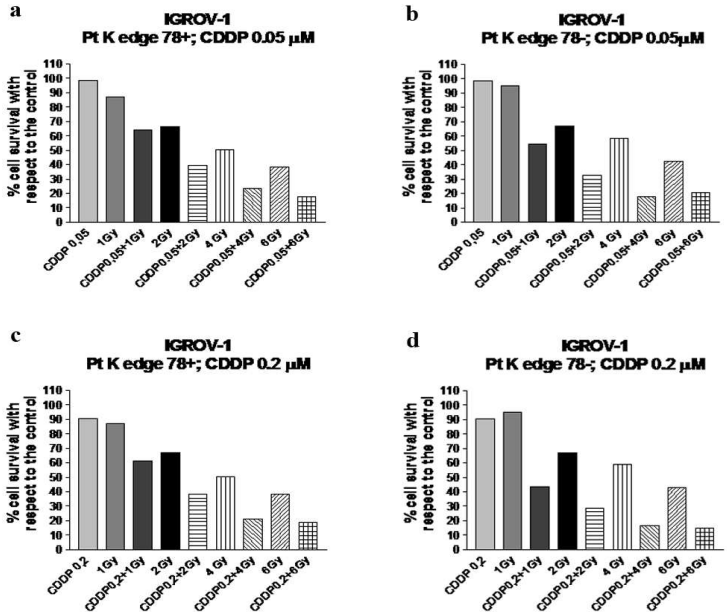

Fig.1 Effect of SR and CDDP combination in IGROV-1 cells evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP 0.05 or 0.2 μM for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge) with a total dose of 1, 2, 4 or 6 Gy. Irradiation with a total dose of 1, 2, 4 or 6 Gy significantly increased cell death with respect to CDDP alone and irradiation alone.

Discussion

Exposure to SR significantly enhances CDDP activity in both A549 and IGROV-1 tumour cell lines of human origin at 1, 2 or 4 Gy at both 78.8 keV (above Pt K-edge) and 78.0 KeV (below Pt K-edge) for both the CDDP concentrations used.

The time point (96 hr after irradiation) chosen for the estimation of the cell survival gave us the possibility to estimate correctly the radiation-induced damage, avoiding the underestimation observed in the proposal MD483. A further experiment will be repeated in the next experimental session planned for December 2010 in order to confirm the results obtained in this serie of measurements.