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: December 2, 2010, 8h00 to: December 3, 2010, 8h00	**Date of report**: April 7, 2011
Shifts: 3	Local contact(s)**: ALBERTO BRAVIN**	*Received at ESRF:*

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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 second serie that took place on December 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. A planned experiment on CSCs cells could not be performed for technical problems. A recuperation day with new beamtime has been allocated on February 2011 in order to perform these experiments.

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 0.2 μM while IGROV-1 were treated with CDDP 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
3. CDDP 0.2 μM	3. CDDP 0.05 μM
4. SR irradiation dose 1Gy	4. SR irradiation dose 1Gy
5. CDDP 0.2 μM +SR irradiation dose 1Gy	5. CDDP 0.05 μM +SR irradiation dose 1Gy
6. SR irradiation dose 2Gy	6. SR irradiation dose 2Gy
7. CDDP 0.2 μM +SR irradiation dose 2Gy	7. CDDP 0.05 μM +SR irradiation dose 2Gy
8. SR irradiation dose 4Gy	8. SR irradiation dose 4Gy
9. CDDP 0.2 μM +SR irradiation dose 4Gy	9. CDDP 0.05 μM +SR irradiation dose 4Gy
10. CDDP 0.2 μM +SR irradiation dose 6Gy	10. 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. Each experimental data point is represented as average value obteined from four replicates. In Fig.2 is reported the mean of the results of two experiments (September and December 2010).

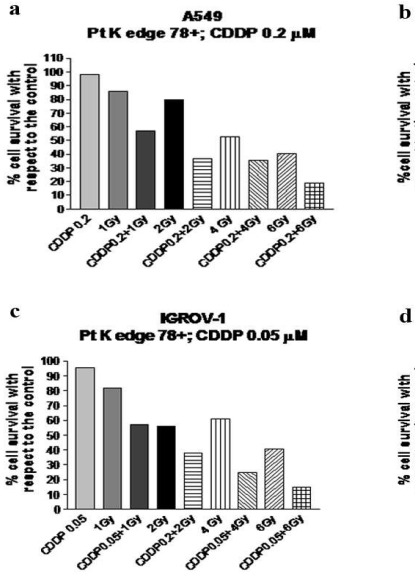

Fig.1 Effect of SR and CDDP combination in A549 and IGROV-1 cells evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP (A549 0.2 μM; IGROV-1 0.05 μM) for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge) with a total dose of 1, 2, 4 or 6 Gy. Irradiation with a total dose of 1, 2 and 4 Gy significantly increased cell death with respect to CDDP alone and irradiation alone.

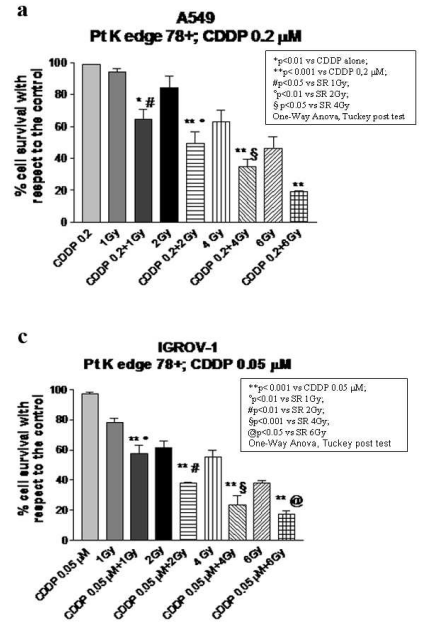

Fig.2 Effect of SR and CDDP combination in A549 and IGROV-1 cells evaluated by Sulforhodamine-B (SRB) cell viability assay. Cells were treated with CDDP (A549 0.2 μM; IGROV-1 0.05 μM) for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge) with a total dose of 1, 2, 4 or 6 Gy. The mean of the results of two experiments (September and December 2010) are reported. Irradiation with a total dose of 1, 2 and 4 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). The results obtained in the September experimenal session are here confirmed thus demonstrating that SR-enhanced CDDP activity in our *in vitro* models. In humans, high-dose CDDP-based treatment is limited by its toxicity (Cavaletti et al., 2008). In particular CDDP induces severe and dose-limiting sensory neuropathy. Based on positive results obtained *in vitro*, new experiments using a reliable *in vivo* animal models (cancer-bearing mice) would now be useful in order to investigate, at the same time, CDDP activity and neurotoxicity. In fact SR-enhanced CDDP activity might allow the use of a reduced dose of CDDP achieving the same antitumour efficacy but minimizing side effects due to the exposure of normal tissues to less toxic doses.

Cavaletti G. *Nat Rev Cancer* 2008; 1p following 71