| ESRF | **Experiment title:** CYTOTOXICITY OF CISPLATIN PLUS SYNCHROTRON RADIATION IN A549 OR IGROV-1 CANCER CELL LINES | **Experiment number**: MD483 |
|---|---|---|
| **Beamline**: ID17 | **Date of experiment**: from: January 23, 2010 8h00 to: January 26, 2010 8h00 | **Date of report**: April 7, 2011 |
| **Shifts:** 6 | Local contact(s)**: ALBERTO BRAVIN** | *Received at ESRF:* |


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**Report:**

This study was a proof-of-concept, exploratory study 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.

***Cell preparation***

A549 or IGROV-1 cells were plated (3000 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 16.5 or 8.5 μM while IGROV-1 were treated with CDDP 7 or 5 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 and 4 Gy. Both cell lines 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 16.5 μM | 2. CDDP 7 μM |
| 3. CDDP 8.5 μM | 3. CDDP 5 μM |
| 4. SR irradiation dose 1Gy | 4. SR irradiation dose 1Gy |
| 5. CDDP 16.5 μM +SR irradiation dose 1Gy | 5. CDDP 7 μM +SR irradiation dose 1Gy |
| 6. CDDP 8.5 μM +SR irradiation dose 1Gy | 6. CDDP 5 μM +SR irradiation dose 1Gy |
| 7. SR irradiation dose 2Gy | 7. SR irradiation dose 2Gy |
| 8. CDDP 16.5 μM +SR irradiation dose 2Gy | 8. CDDP 7 μM +SR irradiation dose 2Gy |
| 9. CDDP 8.5 μM +SR irradiation dose 2Gy | 9. CDDP 5 μM +SR irradiation dose 2Gy |
| 10. SR irradiation dose 4Gy | 10. SR irradiation dose 4Gy |
| 11. CDDP 16.5 μM +SR irradiation dose 4Gy | 11. CDDP 7 μM +SR irradiation dose 4Gy |
| 12. CDDP 8.5 μM +SR irradiation dose 4Gy | 12. CDDP 5 μM +SR irradiation dose 4Gy |

### *Cell survival determination*

24 hr after irradiation cell survival was determined by MTT assay. For the survival assay cells were incubated for 2 hr at 37°C in MTT solution (final concentration 0.5 mg/ml). Formazan crystals formed were dissolved in DMSO and the absorbance was measured at 540 nm using a spectrophotometric microplate reader. Results obtained on IGROV-1 and A549 cells are reported in Fig.1 and Fig.2 respectively. Each experimental data point is represented as average value obtained from four replicates.

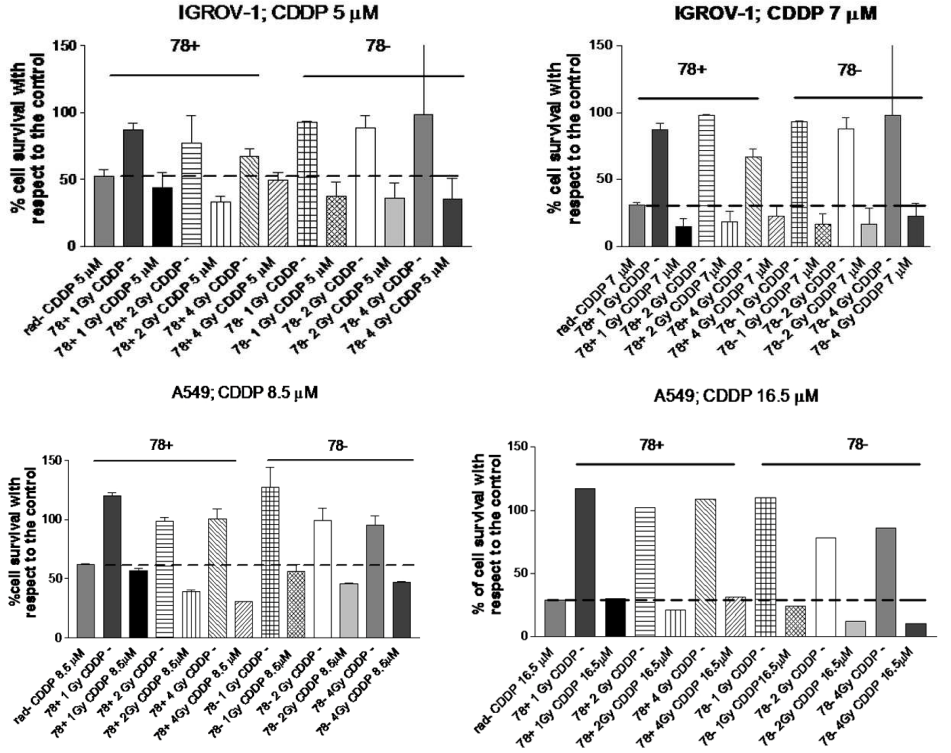


**Fig.1 Effect of SR and CDDP combination on IGROV-1 cells evaluated by MTT cell viability assay.** Cells were treated with CDDP 5 or 7 μM for 24 hours. At the end of treatment cells were irradiated at 78.8 keV (78+; above Pt K-edge) and at 78.0 KeV (78-; below Pt K-edge) with a total dose of 1. 2 and 4 Gv.




**Fig.2 Effect of SR and CDDP combination in A549 cells evaluated by MTT cell viability assay.** Cells were treated with CDDP 16.5 or 8.5 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 and 4 Gy.

### *Discussion*

Exposure to SR significantly enhances CDDP activity in both A549 (non small-cell lung cancer) and IGROV-1 (ovarian cancer) tumour cell lines of human origin. In both cell lines this effect is more evident using the lower CDDP dose (8.5 μM in A549; 5 μM in IGROV-1). In fact with the higher CDDP dose used (16.5 μM in A549; 8.5 μM in IGROV-1) the SR effect is hidden by the CDDP cytotoxicity.

Furthermore the effect of the SR alone seems to be underestimated. Probably it is due to the time point (24 hr after irradiation) chosen for the estimation of the cell survival. In fact after radiation treatment cells destinated to dye can still undergo one or more cell divisions. For this reason it seems necessary that sufficient time is allowed for the cells to die after they are damaged by radiation. The assay duration has to be adapted to the doubling time of the cells in order to correctly estimate the radiation-induced damage.

The possibility to use the SRB assay, which is based on the staining of cellular proteins, has also be evaluated. In fact SRB assay shows a wide linear range with cell number with respect to the MTT test and it is not dependent on mitochondrial activity giving a lower variation between cell lines. Furthermore, compared to MTT, SRB is stable for a long period of time and the experimental procedure can be stopped at several steps during the staining protocol. In addition SRB is a suitable assay for *in vitro* chemoradiation studies. In particular, in the dose range relevant for our study, SRB is comparable in terms of outcome to the clonogenic assay that is generally considered the the optimal test system for *in vitro* radiation studies (Pauwels et al., 2002) even if it is time-consuming, laborious and open to subjective interpretation because of manual counting.

For all these reasons in future experiments we are planning to reduce the CDDP dose in order to treat A549 and IGROV-1 cell lines with a dose of CDDP inducing no more than 5% mortality. Furthermore we will evaluate the survival rate by SRB assay 4 days after irradiation.

Pauwels B, Korst AE, de Pooter CM, Pattyn GG, Lambrechts HA, Baay MF, Lardon F, Vermorken JB. Comparison of the sulforhodamine B assay and the clonogenic assay for in vitro chemoradiation studies. Cancer Chemother Pharmacol. 2003;51: 221-6.