# IV. 2. A Preliminary Study of Proton Therapy Combined with Cisplatin

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A proton beam is expected to have small radiation effects on normal tissue in comparison to a photon beam because of its superior dose distribution, the so-called Bragg peak while a clinical proton beam generating a spread-out Bragg peak have about the same biological effects on tumor cells as an X-ray. Thus, sensitization in proton therapy may provide significant therapeutic advantages over conventional radiotherapy.

The chemotherapeutic agent Cisplatin (cis-diamminedichloro-platinum(II) : CDDP) not only inhibits tumor growth but also give rise to therapeutic enhancement when combined with radiotherapy[1]. The aims of this study were to evaluate therapeutic enhancement for proton therapy combined with CDDP administration in a murine solid tumor model, and to investigate platinum concentration in the treated tumor by means of conventional and submilli PIXE analyses to study a relationship between the CDDP uptake and the enhanced anti-tumor effects caused by the combined treatment. In this report, preliminary results of the CDDP-combined proton therapy and the PIXE analysis of the treated tumors are described.

The experiment was performed using a proton therapy system[2] at Cyclotron and Radioisotope Center (CYRIC), Tohoku University. NFSa fibrosarcoma cells ($5.0\times10^6$) were transplanted into both hind legs of C3H/He male mice aged 10-12 weeks old. When tumor sizes reached a mean diameter of about 10 mm, CDDP was administered intraperitoneally to the mice at a single dose of 10 mg/kg. For proton therapy alone, the tumors in the right hind legs received local proton irradiation without CDDP administration as a single dose of 15 or 30 Gy at a dose rate of 4 Gy/min using an irradiation field 20 mm

in diameter and a 20 mm SOBP. When CDDP and proton therapy were combined, the tumors in the right hind legs were also irradiated locally with a single dose of 30 Gy 1 hour after CDDP administration. The tumor diameters were measured after the treatments using a small animal CT-scanner (Clairvivo® CT, SIMAZU Co. Ltd., Japan) or Vernier calipers.

The conventional PIXE analysis of the tumor tissue were performed using a PIXE analysis system[3] at Nishina Memorial Cyclotron Center (NMCC), Japan Radioisotope Association. The tumors were excised from the mice 6 hours after each treatment and frozen in powdered dry ice. Sample preparation was done by means of a chemical ashing method. The tumor samples were dissolved with nitric acid using a microwave oven. Indium solution (1000 ppm) was added to the samples as an internal standard element. The samples were irradiated using a 2.9 MeV proton beam from an AVF cyclotron at NMCC. Two Si(Li) detectors were used to measure low and high X-ray energy regions separately. A sheet of mylar 500 $\mu$m thickness was used as an absorber in the high X-ray energy measurement.

Platinum distribution in the tumor tissue was investigated by means of submilli PIXE analysis at Tohoku University. We obtained tissue sections by cutting the frozen tumors in a cryostat (-20$^{\circ}$C), and mounted them on 4 mm thick polycarbonate films. Thickness of the section was 250 $\mu$m. The samples were stored at -80$^{\circ}$C until the PIXE analyses. Elemental maps of the samples were obtained from beam scanning with a 3-MeV proton pencil beam. The spot size of the proton beam was about 0.5 mm (FWHM). Details of the submilli camera system have been described elsewhere[4].

Tumor volumes were calculated according to $V=(6/p)abc$, where $a$, $b$ and $c$ are three orthogonal diameters of the tumor. Figure 1 shows time course of tumor volumes after each single treatment. It is found that proton therapy combined with CDDP treatment showed the therapeutic enhancement significantly. The effect of the combined treatment on tumor growth delay may be additive. The conventional PIXE analysis of the tumor sample by the internal standard method resulted in the platinum concentration of 2.6±0.3 ppm. The submilli PIXE results showed that the platinum distribution in the tumor section appears to be almost uniform (Fig. 2), suggesting that CDDP reached hypoxic cell region as well as oxygenated cells in the tumor.

In conclusion, the significant therapeutic enhancement was observed in proton therapy combined with CDDP treatment on the basis of the murine NFSa fibrosarcoma model. The conventional PIXE analysis using the internal standard method showed that

the platinum concentration in the tumor inducing the therapeutic enhancement was 2.6±0.3 ppm. The submilli PIXE analysis of the tumor sections has revealed that CDDP distribution may be almost uniform in the tumor.

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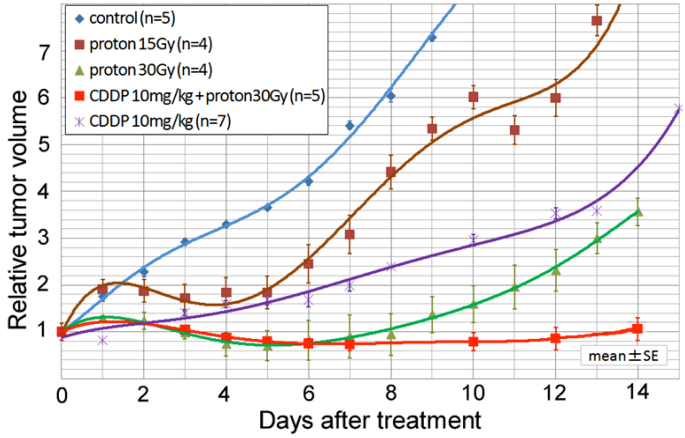


Figure 1. Time course of the tumor volumes of proton therapy alone , CDDP treatment alone and the combined treatment.

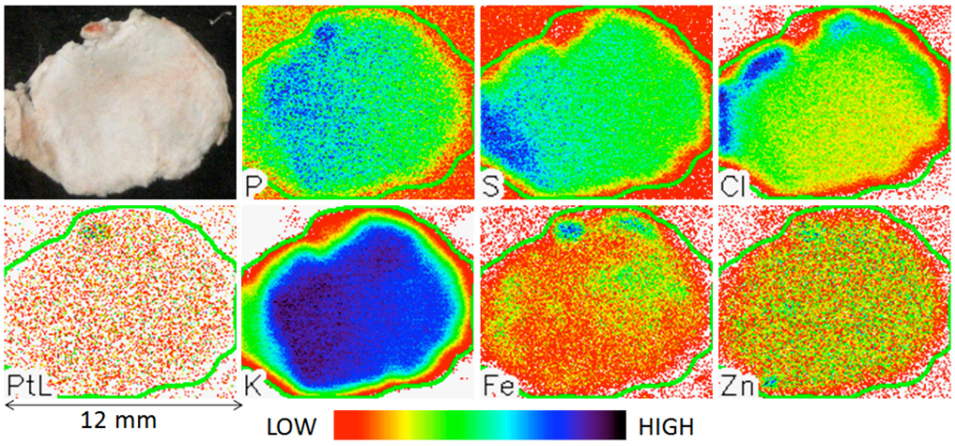


Figure 2. Elemental maps obtained from the submilli PIXE of the tissue section of the CDDP-treated tumor (10 mg/kg).