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In Vitro Thermochemotherapy of Human Osteosarcoma Cells with *Cis*-Dichlorodiammineplatinum (II)

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ABSTRACT

Human osteosarcoma cells (HuO-3N1 cells) and fibroblastic cells with normal human karyotype (HuO-3 cells) were derived from a patient with osteosarcoma. HuO-3N1 cells were more sensitive to heat treatment than HuO-3 cells. The combination of heat and *cis*-dichlorodiammineplatinum (II) (CDDP) exerted synergistic cytotoxity on HuO-3N1 cells, and was much less cytotoxic to HuO-3 cells than to HuO-3N1 cells. Therefore, thermochemotherapy with CDDP may be useful for treatment of human osteosarcoma.

Osteosarcoma is the most common neoplasma, other than myeloma, among the primary malignant tumors of bone. The introduction of adjuvant chemotherapy has greatly improved the survival rate of the patients with osteosarcoma, but the prognosis of the patients is still poor[11].

Hyperthermia has been demonstrated to be lethal in a variety of experimental tumor cells[3,5,7,8]. Tumor cells were more sensitive to heat than normal cells of the same histological type[8]. Thus, hyperthermia is stimulating renewed interest in its clinical application, both alone and in conjunction with ionizing radiation and chemotherapy[2,4].

We now report the selective cytotoxicity of hyperthermia alone and the synergistic cytotoxicity of heat in combination with *cis*-dichlorodiammineplatinum (II) (CDDP) on human osteosarcoma cells (HuO-3N1 cells) *in vitro*.

MATERIALS AND METHODS

Human osteosarocoma cells (HuO-3N1 cells) and fibroblastic cells with normal human karyotype (HuO-3 cells) were derived from a patient with osteosarcoma. The biological properties and growth kinetics of the cells have been reported[12]. In our experiments, HuO-3 cells at the sixth, seventh, and eighth passages and HuO-3N1 cells at the 55th and 56th passages were used. Cells were cultured in Roswell Park Memorial Institute medium 1640 (Nissui Pharmaceutical Co., Tokyo) supplemmented with 10% fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomicin at 37°C in 5% carbondioxide (CO_2) and 95% air.

Growth experiments were performed with methods as described elsewhere [12]. To determine thermosensitivity of cells, a CO_2 incubator was set at 37°C, and another CO_2 incubator was set at the desired temperatures. Cells were seeded in 24-well multiplates (Falcon Plastics, Los Angeles, California, USA), and cultured at 37°C for two days at indicated temperatures after the second day. The number of cells was counted with a Coulter counter (Coulter Electronics, Inc., Hialeah, Florida, USA) every two days. Growth curves were drawn with the average number of cells calculated from triplicate wells. To determine the effect of heat in combination with anti-cancer agents on the growth of

cell, cells were treated with heat alone, or simultaneously treated with heat and anti-cancer agents for three hours the second day after seeding. Growth curves were drawn as described above. To quantify the effect of heat in combination of CDDP on HuO-3N1 cells, we used the regrowth assay method[1, 10] and the colony assay method. In the regrowth assay method, cells were seeded in 24-well multiplates, treated with heat and CDDP for three hours on the second day, and harvested on the sixth day. The one-third cells were reseeded in other 24-well multiplates. The number of cells were counted with a Coulter counter every two days. Regrowth curves were drawn with the average number of cells calculated from triplicate wells. In the colony assay method, 2 x 10^3 and 1 x 10^4 cells were seeded in 60-mm plastic dishes (Falcon) and treated with heat and CDDP for three hours on the second day. Formed colonies were fixed and stained at the 14th day after the treatment. The plating efficiency was calculated from the average number of colonies in triplicate dishes.

Methotrexate (MTX) was obtained from Lederle (JAPAN), LTD., and CDDP was from Nippon Kayaku Co., LTD..

RESULTS

THERMOSENSITIVITY OF CELLS

As shwon in Fig. 1, the number of HuO-3N1 cells markedly decreased in cultivation at 41°C and 42°C, while the number of HuO-3 cells was almost stationary or increased under the same culture conditions.

IN VITRO THERMOCHEMOTHERAPY

The growth rate of HuO-3N1 cells treated with heat and MTX (10^{-8} M or 10^{-6} M) was almost the same as that of the cells treated with MTX alone (data not shown). The number of HuO-3N1 cells increased after the cells were treated with CDDP at 37°C, but decreased after the cells were treated with CDDP at 41°C or 42°C (Figs. 2A, 2B, and 3).

The number of HuO-3 cells increased but that of HuO-3N1 cells decreased after the cells were treated with 1.0 μg/ml of CDDP at 42°C (Fig. 2B). Also, the number of HuO-3 cells increased but that of HuO-3N1 cells decreased, after the cells were treated with 1.0 or 2.0 μg/ml of CDDP at 41°C (Fig. 3). To summarize, HuO-3N1 cells more rapidly proliferated than HuO-3 cells

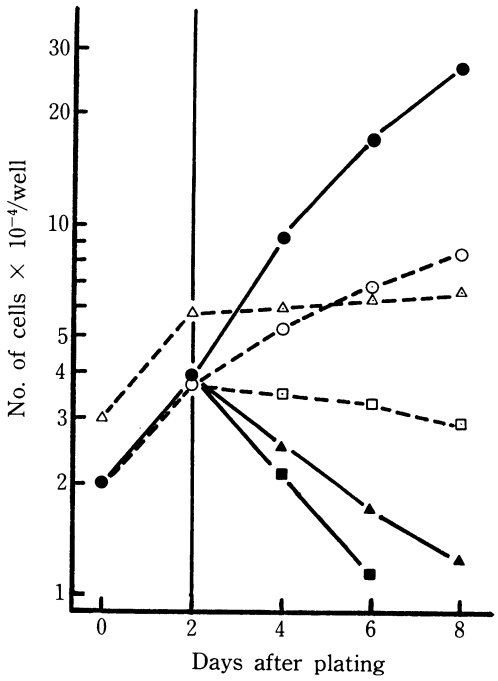


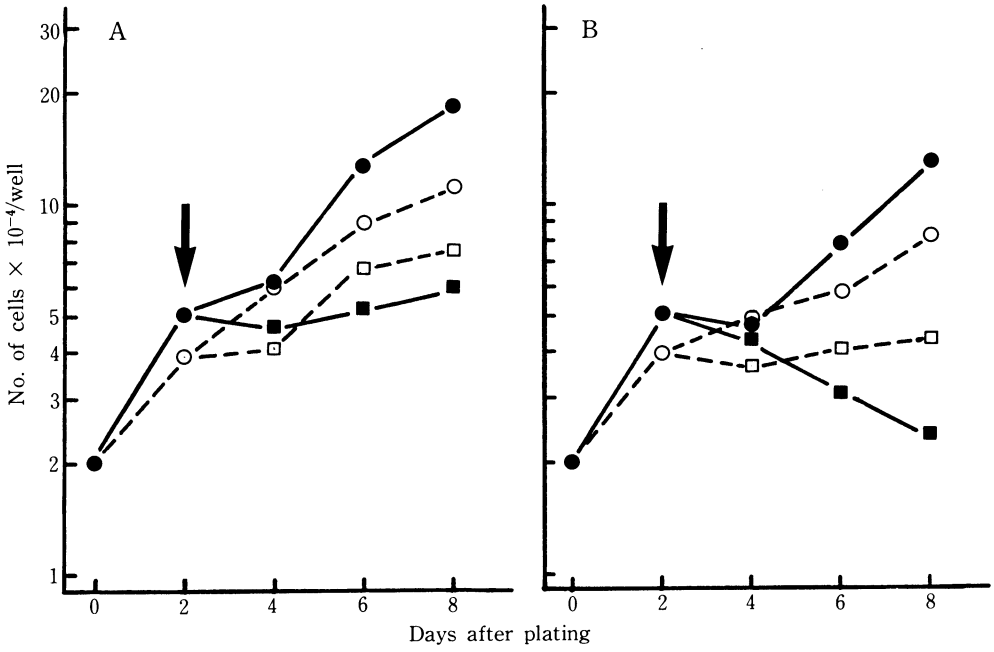
Fig. 1. Growth curves of HuO-3 and HuO-3N1 cells at 37°, 41°, and 42°C, ○ = HuO-3 cells at 37°C; △ = HuO-3 cells at 41°C; □ = HuO-3 cells at 42°C; ● = HuO-3N1 cells at 37°C; ▲ = HuO-3N1 cells at 41°C; ■ = HuO-3N1 cells at 42°C.

after the cells were treated with CDDP at 37°C, but HuO-3N1 cells could not proliferate, even if HuO-3 cells could proliferate, or they proliferated much more slowly than HuO-3 cells after the cells were treated with CDDP at 41°C or 42°C (Figs. 2A, 2B, and 3).

Figure 4 shows regrowth curve of HuO-3N1 cells treated with CDDP at 37°C, and Figure 5 shows CDDP dose-percent survival curves of HuO-3N1 cells calculated from the regrowth curves of the cells treated with CDDP at 37°, 40°, 41°, and 42°C, respectively. Also, Figure 6 shows CDDP dose-percent survival curves of HuO-3N1 cells drawn with the use of the colony assay method. The combination of heat and CDDP exerted synergistic, more than additive, cytotoxic effect on HuO-3N1 cells as shown in Figs. 5 and 6.

DISCUSSION

In a number of reports, it has been demonstrated that hyperthermia has a lethal effect on



Figs. 2A and 2B. Growth curves of HuO-3 and HuO-3N1 cells treated with CDDP at 37°C or 42°C. Figure 2A shows growth curves of cells treated with 0.5 μg/ml CDDP for three hr. ○ = HuO-3 cells treated at 37°C; □ = HuO-3 cells treated at 42°C; ● = HuO-3N1 cells treated at 37°C; ■ = HuO-3N1 cells treated at 42°C. Figure 2B shows growth curves of cells treated with 1.0 μg/ml CDDP for three hr. ○ = HuO-3 cells treated at 37°C; □ = HuO-3 cells treated at 42°C; ● = HuO-3N1 cells treated at 37°C; ■ = HuO-3N1 cells treated at 42°C.

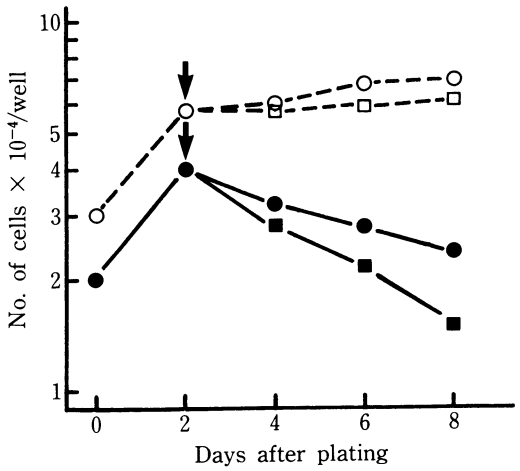


Fig. 3. Growth curves of HuO-3 and HuO-3N1 cells treated with CDDP at 41°C. ○ = HuO-3 cells treated with 1.0 μg/ml of CDDP; □ = HuO-3 cells treated with 2.0 μg/ml of CDDP; ● = HuO-3N1 cells treated with 1.0 μg/ml of CDDP; ■ = HuO-3N1 cells treated with 2.0 μg/ml of CDDP.

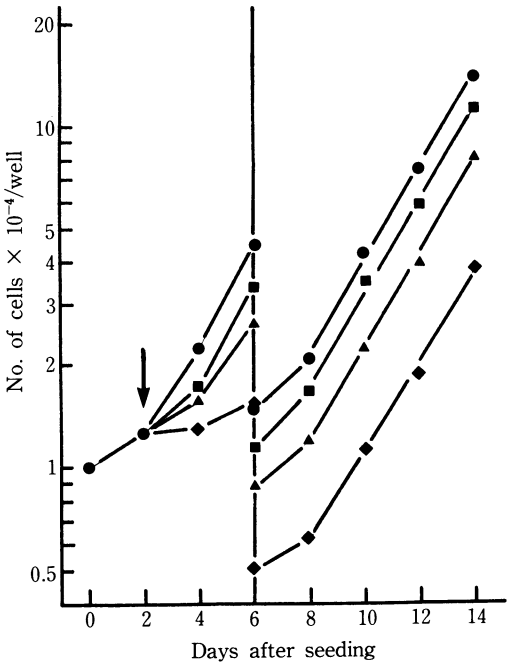


Fig. 4. Regrowth curves of HuO-3N1 cells treated with CDDP at 37°C. ● = control; ■ = 0.5 μg/ml of CDDP; ▲ = 1.0 μg/ml of CDDP; ◆ = 2.0 μg/ml of CDDP.

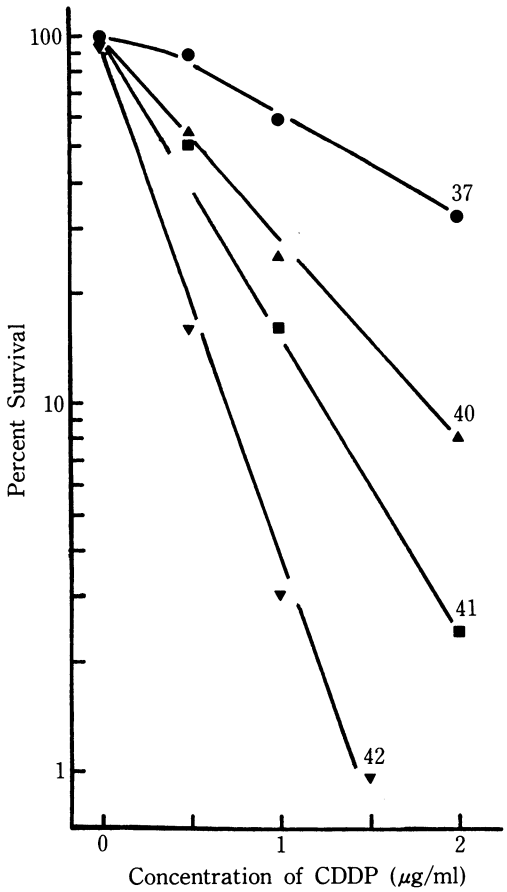


Fig. 5. CDDP dose-percent survival curves of HuO-3N1 cells (with the use of the regrowth assay method). ● = cells treated at 37°C; ▲ = cells treated at 40°C; ■ = cells treated at 41°C; ▼ = cells treated at 42°C.

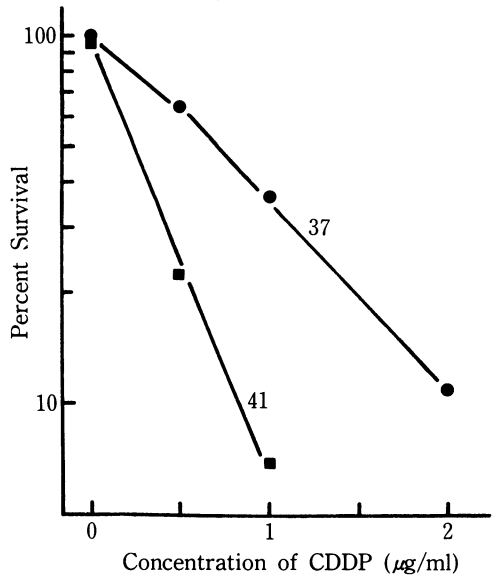


Fig. 6. CDDP dose-percent survival curves of HuO-3N1 cells (with the use of the colony assay method). ● = cells treated at 37°C; ■ = cells treated at 41°C.

experimental tumor cells, and several reports have shown that the combination of heat and some anti-cancer agents exerts synergistic cytotoxicity on tumor cells[3-5,7,8]. But few reports were concerned with bone tumor cells. Hiramoto et al[9] showed that the effect of combination treatment with cyclophosphamide and hyperthermia produced greater reduction on the numbers of osteosarcoma cells than did either treatment used alone *in vivo*.

In a previous report[8], the effect of the heat on tumor cells was compared to that on normal cells. However, in the report, the cells derived from normal adult and embryonal tissues were used as normal cells. In this report, osteosarcoma cells (HuO-3N1 cells) were compared to fibroblastic cells with normal human karyotype (HuO-3 cells) derived from the same patient with osteosarcoma from which HuO-3N1 cells were derived. HuO-3N1 cells were much more sensitive to heat than HuO-3 cells, and the combination of heat and CDDP exerted synergistic cytotoxicity on HuO-3N1 cells. Now, noninvasive deep-heating methods are available[6]. Therefore, thermochemotherapy with CDDP will be useful for treatment of osteosarcoma.

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