



Delay in administration of CDDP until completion of AGM-1470 treatment enhances antimetastatic and antitumor effects

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Abstract

The efficacy of *cis*-diammine dichloroplatinum (CDDP) therapy in combination with continuous administration of angiogenesis inhibitor *o*-(chloroacetyl-carbamoyl) fumagillol (AGM-1470) was evaluated experimentally using a transplantable rat osteosarcoma line previously established in our laboratory. AGM-1470 (2.5 mg/kg body weight/week) was administered by Alzet osmotic pumps for 2 weeks starting from 7 days after tumor implantation and CDDP (1.25 mg/kg) was given on days 21 and 24. The number of lung metastatic nodules was counted and the wet weights of the primary tumors were measured 5 weeks after tumor implantation. Values with administration of CDDP 3 days after discontinuation of AGM-1470 were significantly lower than when the two agents were coadministered ($P < 0.05$). This animal model should facilitate optimization of the timing of combination therapy.

Introduction

Osteosarcomas readily spread through the bloodstream to the lungs, resulting in metastases that make the prognosis poor after noncurative or even curative treatments. To prevent the development of secondaries, it is, therefore, important to give optimal chemotherapeutic treatment. The angiogenesis inhibitor, AGM-1470, a synthetic analogue of fumagillin is an attractive drug because of its antitumor and antimetastatic effects against various tumors [1–7]. However, it is hypothesized to be selectively cytotoxic toward endothelial cells [8] and exponential relapse may occur after treatment. Therefore, combination with conventional therapeutics might be necessary for eradication of primary tumors and metastatic nodules.

Combinations of AGM-1470 and agents targeting tumor cells themselves may show additive or synergistic effects [9–11], but Devineni et al. have reported that coadministration with a cytotoxic agent results in limited antitumor effects because the capillary permeability is reduced [12].

In the present experiments, we investigated the effects of CDDP administration, either together with AGM-1470, or after a 3-day interval. The study casts some light on the effect of this combination chemotherapy.

Materials and methods

Thirty-five male Fisher 344/NS1c rats (Shizuoka Laboratory Animal Center, Shizuoka), 5 weeks old at the commencement of the experiment, were used to assess the effects of therapy on primary transplanted tumor growth and lung metastasis. The animals were housed in plastic cages with wood chip bedding in an air-conditioned room at 22 °C and 60% humidity under a daily cycle of alternating 12-h periods of light and dark, and given Oriental MF diet (Oriental Yeast, Tokyo) and water *ad libitum*. After a 1-week acclimatization period, when the rats weighed approximately 120 g each, they received implants of the S-SLM osteosarcoma, which has a high rate of metastasis to the lung. Five weeks after tumor implantation, the recipient rats were euthanized under ether anesthesia.

Chemicals

AGM-1470 was kindly supplied by Takeda Chemical Industries Co. Ltd, Osaka and prepared for administration by being dissolved in 100% ethanol at a concentration of 5 mg/ml. CDDP liquid at a concentration of 0.5 mg/ml was obtained from the Nippon Kayaku Co. Tokyo.

Tumor transplantation

The S-SLM is a transplantable osteosarcoma showing a high incidence of spontaneous lung metastasis. It was originally derived from a spontaneous osteosarcoma (S-OS) by *in vivo* selection. Details regarding the methods of production and transplantation of tumors have been described previously

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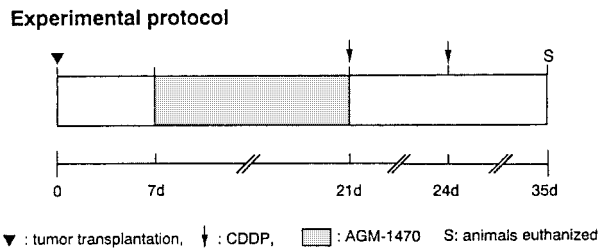


Figure 1. Experimental protocol used to assess the effects of CDDP and/or AGM-1470 on osteosarcoma growth and lung metastasis in rats.

[13, 14]. Lung metastatic nodules, approximately 3 mm in diameter, were transplanted into the right subcutaneous back space of syngeneic rats. All procedures were performed under aseptic conditions.

Experimental procedure

The basic experimental procedure is shown in Figure 1. To evaluate the chemotherapeutic efficacy in terms of the growth of subcutaneous tumors and formation of lung metastatic nodules, rats were divided into 7 groups after receiving transplants. Group 1 received no further treatment; group 2 was given AGM-1470 by Alzet 2002 osmotic pumps (ALZA Co., Palo Alto, CA) at a dose of 2.5 mg/kg body weight/week starting 7 days after the transplantation for 14 days; groups 3 and 4 received the same treatment as group 2 followed by single intravenous injections of CDDP at a dose of 1.25 mg/kg at 21 and 24 days, respectively; groups 5 and 6 received the transplantation without AGM-1470 and the same dose of CDDP at 21 and 24 days, respectively. Group 7 was given the vehicle (100% ethanol) alone for 2 weeks by osmotic pumps. Alzet 2002 osmotic pumps were inserted into the left subcutaneous back space of the rats. Each group consisted of five rats. Before starting the experiments, we confirmed that the 100% ethanol used as a vehicle did not affect the pump reservoirs by ALZAID (Chemical Compatibility Test Kit, ALZA Co., Palo Alto, CA).

Tumor size and body weight were measured once a week. Caliper measurements of perpendicular diameters were used to calculate tumor volumes in cubic millimeters by use of the following formula: (longest diameter) \times (shortest diameter)² \times 0.5. The wet weights of the primary tumors were also determined and visible lung metastatic nodules were counted after autopsy.

Statistical analysis

The statistical significance of differences between groups was determined by applying the Mann-Whitney test for numbers of the metastatic nodules and the Student's *t*-test for primary tumor volumes and weights.

Results

Suppression of tumor growth was observed after 2-weeks' administration of AGM-1470, followed by rapid regrowth 3 days after discontinuation of AGM-1470 in group 2 (see

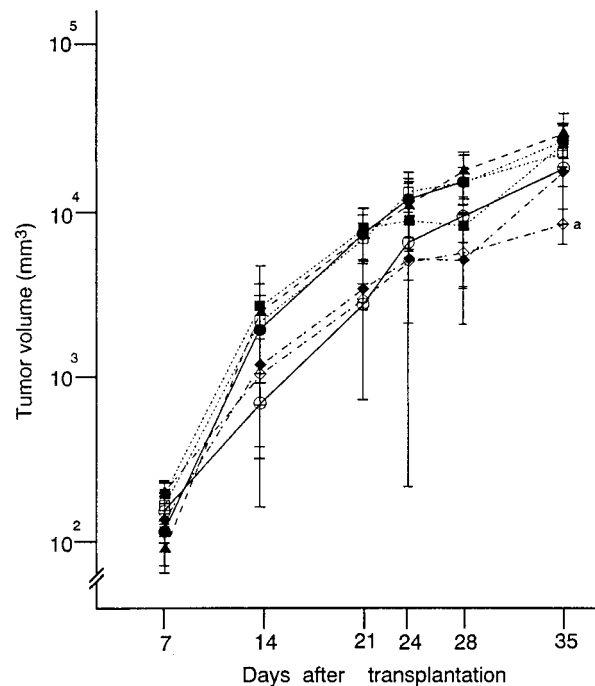


Figure 2. Growth curves of subcutaneous tumors in rats receiving AGM-1470 and/or CDDP. Group 1: ●, group 2: ○, group 3: ◆, group 4: ◇, group 5: ■, group 6: □, group 7: ▲. Values are means with S.D.s represented by vertical bars. ^aSignificantly different from group 3 ($P = 0.03$).

Figure 2). In group 3, similar growth was observed even though CDDP was injected at the time of discontinuation of AGM-1470 (at day 21). The average final tumor volume in group 3 was approximately the same as that in group 2 receiving AGM-1470 alone. In group 4, the administration of CDDP 3 days after discontinuation of AGM-1470 inhibited tumor growth throughout the subsequent period of the experiment. In groups 5 and 6, the administration of CDDP caused the tumors to be a little smaller 1 week and 4 days thereafter, respectively, but they showed rapid growth during the final week.

The final inhibition rate in group 3 (49.6%) was approximately the same as that of groups 2, 5 and 6, despite the coadministration of CDDP and AGM-1470. The final inhibition rate in group 4 (83.4%) was obviously higher than in the other treated groups (Table 1), with final primary tumor weights being significantly lower compared with groups 2, 3, 5 and 6.

The mean number of lung metastatic nodules in group 2 (272.6 ± 61.3) was a little less than that in the control group (over 300) (Table 2). In group 3 the mean number of lung metastatic nodules (7.2 ± 5.9) was decreased but not significantly compared with groups 5 and 6 (treated with CDDP alone). In group 4 the mean number of lung metastatic nodules (1.4 ± 2.2) was clearly lower than those in groups 3, 5 and 6.

In group 7, the administration of the 100% ethanol for 2 weeks by Alzet 2002 pumps had no inhibitory effects against both the tumor growth and the formation of the lung metastatic nodules.

Table 1. Chemotherapeutic effects of CDDP alone, together with or after AGM-1470 treatment, on primary transplanted tumors in rats.

Group no.	Tumor weight (gm)		Inhibition rate (%)
	Mean \pm S.D. (range)		
1	N.D.		
2	29.1 \pm 9.9 ^a (14.2–37.2)		50.8
3	21.6 \pm 8.9 ^b (6.6–29.8)		49.6
4	10.7 \pm 2.4 (7.8–13.0)		83.4
5	32.0 \pm 9.4 ^c (19.1–41.7)		45.0
6	25.1 \pm 11.2 ^d (16.1–44.4)		46.1
7	N.D.		–37.2

$$\text{Relative tumor volume (five weeks after transplantation)} = \frac{\text{Final tumor volume (five weeks after transplantation)}}{\text{Initial tumor volume (one week after transplantation)}} = \left(1 - \frac{\text{RMTV in treated group}}{\text{RMTV in control group}}\right) \times 100 (\%)$$

^aSignificantly different in groups 4 and 2; $P = 0.004$.

^bSignificantly different in groups 4 and 3; $P = 0.03$.

^cSignificantly different in groups 4 and 5; $P = 0.001$.

^dSignificantly different in groups 4 and 6; $P = 0.02$.

N.D. – not measured.

Table 2. Chemotherapeutic effects of CDDP and/or AGM-1470 on lung metastasis in rats.

Group no.	Metastatic foci		Metastatic incidence
	Mean \pm S.D. (range)		
1	Over 300 (over 300)		3/3 ₂
2	272.6 \pm 61.3 ^a (163–over 300)		5/5
3	7.2 \pm 5.9 ^b (2–17)		5/5
4	1.4 \pm 2.2 (0–5)		2/5
5	20.6 \pm 23.7 ^c (3–59)		5/5
6	29.9 \pm 27.1 ^c (2–68)		5/5
7	275.0 \pm 50.0 ^d (200–over 300)		4/4 ₁

^aSignificantly different from groups 2 and 3; $P = 0.01$.

^bSignificantly different from group 4; $P = 0.05$.

^cSignificantly different from group 4; $P = 0.02$.

^dNot significantly different between groups 1 and 7.

₂ and ₁: spontaneous mortality.

Discussion

AGM-1470 is a promising drug because of its obvious antitumor and antimetastatic effects against various human and rodent tumors [1–7]. We earlier reported its inhibition of osteosarcoma development and showed this not to be due to direct effects on osteosarcoma cells because their I.C.₅₀ value by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (4 ng/ml) was elevated compared with that of rat endothelial cells [6]. The administration of AGM-1470 at 2.5 mg/kg body weight/week for 4 weeks showed antitumor and antimetastatic effects comparable to those of intermittent administration at 20 mg/kg/body weight three times per week (unpublished data). However, exponential relapse may occur after AGM-1470 discontinuation because of its selective inhibition of endothelial cell proliferation [8]. Therefore combination chemotherapy of angiogenesis inhibitors and cytotoxic agents has been considered as a new therapeutic strategy.

However, for conventional therapeutics to be effective, it is necessary that they reach the malignant cells of a tumor [15]. Teicher et al. reported increased cytotoxicity of CDDP toward EMT-6 cells after exposure to AGM-1470 (5 μ M, 24 h) [16] and other authors have also described additive or synergistic effects with cytotoxic agents or hyperthermia [9–11]. However, Devineni et al. recently reported reduction in efficacy against primary tumor cells because of decreased tumor uptake of TMZ [12].

In the present study, when CDDP was given at the end of the period of AGM-1470 treatment, the antimetastatic effects were increased but antitumor effects were not in comparison with the CDDP injection alone in line with the effects reported by Devineni et al. [12]. However, tumor growth was also inhibited after CDDP injection on day 24 and metastatic foci were even further suppressed. The regrowth observed immediately after AGM-1470 withdrawal is highly consistent with the recovery of HUVE cells in culture [17] and the extremely short plasma half life of the drug (range 5.5–10 min) [13]. Nishimura et al. also reported that tumor angiogenesis occurs 3 days after hyperthermia treatment [19] and it is well known that malignant tumors begin exponential growth once they are vascularized [20, 21]. The recovery 3 days after discontinuation of AGM-1470 could thus paradoxically have allowed the drug to be more effective, as earlier reported [12].

With regard to formation of lung metastases by this osteosarcoma line, no metastases were observed when primary transplanted tumors were removed 5 days after transplantation but at 7 days secondary growths had already been seeded (unpublished data). Krotolica and Ludlow reported ovarian carcinoma cells maintain their invasiveness under hypoxic conditions [22] and Schwickert found a high risk of metastasis in human cervical cancer under hypoxic conditions [23]. In this study, the metastatic potential in group 2 might thus have been maintained during the continuous treatment with AMG-1470 started 7 days after tumor transplantation. Therefore the data implies that the CDDP injection was effective at inhibiting the development of secondary growths as metastatic nodules in treated groups.

Continuous treatment with AGM-1470 has been reported to prolong the dormant phase of metastases [24] and lung metastatic nodules would be expected to exhibit rapid growth after removal of any angiogenesis block by AGM-1470, similar to primary tumors. With delayed CDDP administration, the lung metastatic nodules would have entered a rapid growth phase and this difference between the angiogenesis states of the tested groups might explain the enhanced inhibition observed similar to that for tumor growth.

However, some other factors, for example, a synchronized passage through the cell cycle after discontinuation of AGM-1470 could have played a role. We did not investigate any interaction between AGM-1470 and CDDP nor the effects combined with other chemotherapeutics (doxorubicin or ifosfamide). Further investigations are necessary to obtain more informations about combination therapy.

In groups 3 and 4, the body weights of rats tended to decrease during the administration of AGM-1470 but after discontinuation of AGM-1470 the body weights rapidly recovered to control level and, consequently, there were no significant differences among groups 1, 3 and 4 at euthanasia. No toxic death was observed during this experimental period.

In summary, this study provided evidence that antitumor and antimetastatic effects are enhanced when administration of CDDP is delayed until 3 days after completion of a 2-week AGM-1470 therapeutic regimen.

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