Cancer Chemother Pharmacol (2000) 46: 423–426

ORIGINAL ARTICLE

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Combined effects of the orally active cisplatin analog, JM216, and radiation in antitumor therapy

Received: 20 December 1999 / Accepted: 24 May 2000

Abstract *Purpose*: We evaluated the orally administered platinum agent, JM216, in combination with ionizing radiation both in vivo and in vitro against human tumor cells. *Methods*: H460 human lung carcinoma cells were used as a subcutaneous xenograft in nude mice. JM216 (30 mg/kg) was administered orally, and radiation treatments (2 Gy) were given 1 h after JM216 delivery for five consecutive days. For in vitro analysis, attached H460 cells were treated with JM216 (15 μ*M*) for 1 h and then irradiated. Cells were rinsed 20 min later, and survival was determined by clonogenic assay. *Results*: Tumor growth delay measurements showed that the combination of JM216 and radiation was additive in vivo, with an enhancement ratio of 1.24. In vitro clonogenic survival experiments demonstrated a dose enhancement ratio of 1.23. Isobologram analysis showed that this interaction was also additive. *Conclusions*: These data demonstrate that the combination of JM216 and fractionated radiotherapy is more effective against human lung cancer xenografts than either agent alone, and the in vivo results were supported by those observed using an in vitro system with the same tumor cell line.

Key words Radiation · Cisplatin · JM216 · Lung cancer · Radiotherapy

Introduction

Concomitant radiotherapy and chemotherapy is commonly used to treat non-small-cell lung cancer. Platinum agents such as cisplatin and carboplatin are routinely used for this purpose since they can enhance the effects of radiation. The first in vivo demonstration of this effect was performed using the P388 mouse leukemia model, in which combined cisplatin and radiation increased the lifespan of tumor-bearing mice more effectively than either agent alone [1]. Several studies have been reported since then which have shown an enhancement of radiation effects both in vivo and in vitro by cisplatin and carboplatin [2, 3, 4]. The recently developed cisplatin derivative, bis-ace tato-ammine-dichloro-cyclohexylamine platinum(IV) (JM216), has cytotoxic mechanisms similar to those of cisplatin [5], but has been shown to have less-toxic side effects than cisplatin in clinical trials [6]. Since JM216 is administered orally, it can be given on a daily basis, which is useful for fractionated radiotherapy.

JM216 has been evaluated without radiotherapy in both in vivo preclinical studies and human clinical trials. JM216 has shown antitumor selectivity far superior to that observed for cisplatin, carboplatin, or tetraplatin against murine plasmacytoma [6]. Against human ovarian carcinoma xenografts and murine sarcoma, JM216 shows antitumor activity similar to that of carboplatin [7]. Antitumor schedule dependency for oral JM216 has been demonstrated using two tumor model systems, with optimal activity using daily times-five dosing [8]. Although phase I clinical trials have shown that single-dose or twice-daily oral administration of JM216 is not well tolerated, daily times-five dosing with about 100 mg/m^2 per day appears to have less-toxic side effects and is being recommended for phase II trials [9].

In this study, the combined effects of JM216 and radiotherapy were investigated in vivo using a human lung cancer xenograft in nude mice. We also sought to determine whether JM216 can enhance the effects of radiation in the same cell line using a clonogenic assay.

This work was funded by a grant CA-82117-02 and a grant from Bristol-Myers Squibb

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Materials and methods

Cell culture

NCI-H460 human large-cell lung carcinoma cells were obtained from the American Type Culture Collection (ATCC). H460 cells were cultured at 37 °C in an atmosphere containing 5% CO_2 in RPMI-1640 medium (Gibco BRL, Gaithersburg, Md.) containing 10% fetal bovine serum (Gibco), 50 U/ml penicillin (Gibco) and 50 μg/ml streptomycin (Gibco). Cells were passaged twice-weekly using 0.05% trypsin.

Mice and tumor inoculations

Human NIH-H460 cells were used as a xenograft model in female athymic nude mice (*nu*/*nu*, 5–6 weeks old). H460 cells (3×10^6) were plated in 75-mm^2 flasks 2 days prior to inoculation, and the medium was replaced with fresh medium 24 h later. At 48 h (about 80% confluency), the cells were trypsinized, resuspended in growth medium, and counted. Cells were then centrifuged for 5 min at room temperature, and resuspended in PBS at 2×10^7 cells/ml. A suspension of 2×10^6 cells in a 0.1 ml volume was injected subcutaneously (s.c.) into the left posterior flank using a 27.5-gauge needle. Tumors were allowed to grow for 7 days before treatment.

Drug and radiation treatments

Fresh stock solutions of JM216 (Bristol-Myers Squibb) were made up before each experiment by suspending the compound in peanut oil (Planters) at the appropriate concentration. Drug doses (30 mg/kg) were given orally in a volume of 0.2 ml using a 20-gauge 1.5-inch animal feeding needle 1 h prior to radiation, with five mice per treatment group. Tumors on the flanks of mice were irradiated with 2 Gy for five consecutive days using an Eldorado-8 ^{60}Co irradiator. The dose rate was 1.18 Gy/min at a source skin distance of 56.5 cm. The nontumor parts of the mice were shielded using lead blocks. Mice were irradiated using restraining devices constructed from 50 ml conical polyethylene tubes taped to a styrofoam pad.

Tumor measurements and data analysis

Two perpendicular diameters were measured three times weekly after treatment using a vernier caliper. Results were evaluated based upon the formula: volume $= 0.4 \times ab^2$ where 'a' is the longer dimension, and 'b' is the shorter dimension. Experiments were started when tumors reached an average volume of about 0.3 cm^3. Growth delay time (GD) was calculated as the time for treated tumors to triple in volume minus the time for control tumors to triple in volume, with t = 0 defined as the first day of treatment. The enhancement factor (EF) was then determined as follows [10]:

$$EF = (GD_{JM+xRT} - GD_{JM})/GD_{xRT}$$

where JM represents JM216 treatment, and xRT represents radiation treatment. Tumor volume fractions were also statistically compared using the two-tailed Student's *t*-test [11].

In vitro survival analysis

Log-phase cells were trypsinized, counted, and plated in triplicate per data point into 25-mm^2 flasks. JM216 stock solutions were made in DMSO (Sigma) at 10 m*M*, and diluted into medium immediately before experiments. The final DMSO concentration in the drug medium was 0.2% or less. Sham treatment with DMSO had no effect upon radiation survival. Cells were treated with 15 μ*M* JM216, incubated at 37 °C for 1 h, irradiated with ^{60}Co gamma rays, then returned to 37 °C for 20 min. Cells were then rinsed with phosphate-buffered saline (PBS), and returned to 37 °C for 9 days. Colonies were fixed with 3:1 methanol/acetic acid and stained with 0.5% crystal violet (Sigma) in methanol. Colonies were counted by eye, with a cut-off of 50 viable cells. The surviving fraction (SF) was calculated as mean colonies/(cells inoculated × plating efficiency). The dose enhancement ratio (DER) was calculated as the dose (Gy) for radiation alone divided by the dose (Gy) for radiation plus drugs (normalized for drug toxicity), at an SF of 0.10. Isobologram analysis was performed at an isoeffect of SF = 0.10 by the method of Steel and Peckham [12].

Results

The results of in vivo studies with JM216 and radiotherapy are shown in Fig. 1. Three independent experiments were performed, and the mean values are shown. Tumor GD values based upon tumor volume tripling time were calculated (Table 1), and the EF derived from Fig. 1 was 1.24. Thus, the combination of 30 mg/kg JM216 and 2 Gy radiation delivered daily for five consecutive days was more effective than either agent alone. The fraction of control tumor volumes for day 7 and day 9 is given in Table 2. The expected additive fraction for the combined JM216 plus radiation group on day 7 would be $0.74 \times 0.78 = 0.58$; additivity for the combined treatment group on day 9 would be 0.54. The experimentally determined fractions shown in Table 2 are slightly lower than these values. However, using a two-tailed Student's *t*-distribution, the measured fractions at both time-points were not significantly different from the expected additive fractions at the 99% confidence interval ($P = 0.01$). Thus, JM216 and radiation combined in an additive fashion in this particular system, even though the ER was greater than unity.

Figure 2 shows the results of three independent experiments using JM216 plus radiation in vitro against

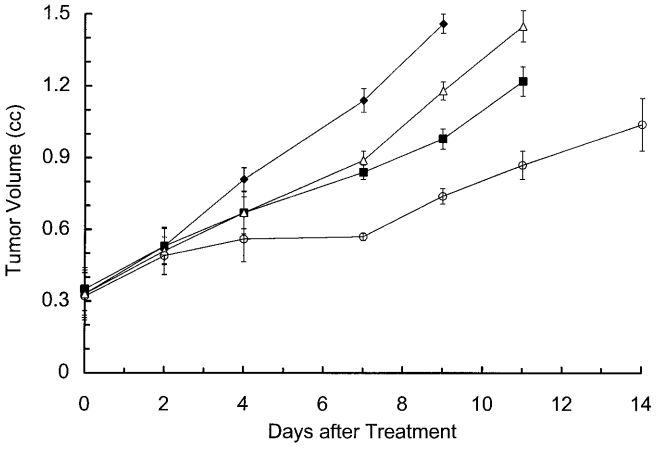

Fig. 1 Combined effects of JM216 and radiotherapy on H460 human lung cancer xenograft growth delay using a fractionated regimen. Mice were given oral doses of 30 mg/kg JM216 and/or irradiated 1 h later with 2 Gy on five consecutive days. Tumor volume was measured three times weekly for 2 weeks. *Error bars* represent the standard error of the mean (±SEM) from three independent experiments. Each experiment contained five mice per treatment group (◆ untreated control, ■ radiation alone, △ JM216 alone, ○ JM216 plus radiation)

Table 1 Tumor growth delay (GD) values calculated from the data in Fig. 1 based upon tumor tripling time. Values are means ± SEM from three independent experiments

Treatment group	Mean growth delay (days)
Radiation only (2 Gy in five fractions)	3.9 ± 0.4
JM216 only (30 mg/kg in five fractions)	2.1 ± 0.9
Radiation + JM216	6.9 ± 1.4

Table 2 Fraction of control tumor volumes for day 7 and day 9 measurements. Values are mean ± SEM tumor volumes (normalized by control volumes) from three independent experiments. Student's *t*-test was then used to compare the measured fraction for both agents (4th row) to the expected fraction for additivity (2nd row × 3rd row)

Treatment group	Fraction of control volume	
	Day 7	Day 9
Untreated control	1.0 ± 0.043	1.0 ± 0.027
Radiation only	0.74 ± 0.026	0.67 ± 0.029
JM216 only	0.78 ± 0.034	0.81 ± 0.025
Radiation + JM216	0.50 ± 0.013	0.51 ± 0.023

H460 human lung carcinoma cells. The radiation DER for 15 μ*M* JM216 at an SF of 0.10 was 1.23. Based upon isobologram analysis at an isoeffect of SF = 0.10 (not shown), which is a more rigorous test of superadditivity, the combination of 15 μ*M* JM216 and radiation in vitro was also additive in H460 lung cancer cells.

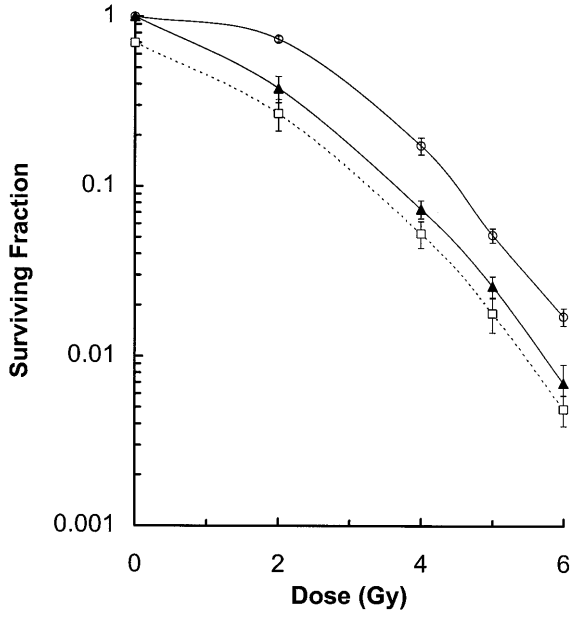

Fig. 2 Clonogenic survival curves for H460 cells treated with radiation plus JM216. Attached cells were treated with JM216 for 90 min with radiation given at t = 60 min. *Error bars* represent the standard error of the mean (±SEM) of three independent experiments. Each experiment had three replicate flasks per data point (○ radiation only; ▲ radiation plus 15 μ*M* JM216, normalized for the killing by drug alone, SF = 0.71 ± 0.04; □ radiation plus 15 μ*M* JM216, not normalized)

Discussion

The data indicate that the combination of JM216 and radiation was more effective against human tumor xenografts than either agent separately. One study using the RIF-1 tumor model in mice has shown that superadditivity occurs when cisplatin is delivered on a fractionated schedule (5 × 2.4 mg/kg per day) immediately before radiation treatment (5 × 1000 rad/day) [4]. Another study has shown an additive effect between cisplatin and radiation when mice are given cisplatin followed by radiation treatment 45 min later. Other platinum analogs which were complexed with dye molecules have been shown to act as radiosensitizers in vivo using the Lewis lung carcinoma model in mice; the effects were similar to that of cisplatin [13]. Intratumoral delivery of cisplatin has been previously studied using polymer rod delivery systems [2]. In these studies the DERs varied from 1.1 to 1.7. The ERs for the in vivo and in vitro experiments were very close in our study. However, the raw data (SF vs fraction of control tumor volume) indicate that the in vitro interaction was more significant. This is due to the fact that a ratio of radiation doses was used for the in vitro factor, while a ratio of GD values was used for the in vivo analysis.

The cisplatin analog, JM216, has been previously shown to act as a radiosensitizer in murine oxic cells in vitro [14]. Cisplatin has been shown to inhibit sublethal damage repair (SLDR) in vivo, as evidenced by the ability of murine tumors to recover from fractionated radiation doses [15]. Both cisplatin and carboplatin inhibit SLDR in vitro [3, 4], as defined by split dose recovery experiments. Thus, the in vitro interaction between JM216 and radiation may involve inhibition of sublethal damage repair following DNA damage by ionizing radiation.

In conclusion, the orally administered cisplatin analog, JM216, was able to enhance the effects of radiation both in vivo and in vitro using a human lung cancer model. The interaction between the agents in vivo was additive, according to statistical analysis, but this study shows that there may be an advantage to using both agents together as opposed to either agent separately. These preclinical data show that a cisplatin analog which is orally active may have clinical utility in combination with a fractionated radiotherapy regimen against non-small-cell lung cancer.

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