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Radiosensitization of FM3A Cells by 5-Fluorouracil Plus Cisplatin

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Summary. **The effects of radiation in combination with either 5-fluorouracil (5-FU), cisplatin (CDDP), or both on FM3A cell survival were evaluated *in vitro*. Cell survival following treatment with the drugs and/or radiation was determined by colony formation assays. Prolonged exposure of 5-FU as well as CDDP was very effective in killing cells. The combination of radiation with 24-hour continuous exposure of the drugs resulted in radiosensitization, while 1-hour pulse exposure of CDDP combined with radiation did not enhance cell killing, indicating that radiosensitization by CDDP may require a longer duration of drug exposure following irradiation. In addition, concurrent continuous exposure of 5-FU and CDDP in combination with radiation resulted in a greater radiosensitizing effect.**

Key words—radiation, 5-fluorouracil, cisplatin, FM3A cells, radiosensitization.

INTRODUCTION

In current cancer treatment, various combinations of antineoplastic agents and radiation have been used to achieve better local control and survival. Although numerous studies have been undertaken to obtain improved treatment results by chemoradiotherapy, it is still unclear which agents should be used and how they are to be combined with radiation. Recently, the combination of radiation with the concurrent administration of 5-fluorouracil (5-FU) and cisplatin (CDDP) has been employed for various human tumors such as cancer of the head and neck,[1] cancer of the esophagus,[2,3] cancer of the anus,[4] and gynecological malignancies.[5] In these regiments 5-FU and CDDP have been administered more often as a continuous intravenous infusion to improve local control and simultaneously to diminish the toxicity of the drugs, caused by an intravenous bolus infusion.

5-FU is known as a radiosensitizer when combined with radiation. *In vitro* studies have suggested that optimal radiosensitization can be obtained by prolonging drug exposure time beyond the cell cycle time.[6,7] CDDP has been also reported to enhance the cytotoxicity of radiation.[8-10] Therefore, there is a sound rationale for employing 5-FU and CDDP in conjunction with radiation concurrently, based not only on the radiosensitizing properties of the two agents, but on the phenomenon known as biochemical modulation in which alteration in the metabolism of 5-FU by biochemical means, including CDDP, can enhance the cytotoxicity of 5-FU. However, preclinical data available on the combination of these modalities have been relatively limited.

In this study the effect of radiation combined with either 5-FU, CDDP or both was examined *in vitro* under aerobic conditions. These experiments were performed by exposing exponentially growing cells to the drugs and radiation, and then assaying for their colony forming ability. An attempt was made to simulate clinical situations often encountered.

MATERIALS AND METHODS

FM3A cells were derived from a C3H mouse mammary carcinoma. They were grown in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum and maintained at 37°C in a humidified atmosphere. All experiments were performed using exponentially growing cells ($2\text{-}3 \times 10^5$)

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with a doubling time of 12.5 h.

5-FU and CDDP were separately diluted in MEM to obtain appropriate concentrations indicated in the figure legends, which were used immediately after preparation. In cases where radiation exposure was required, 5-FU alone, CDDP alone or both in combination were added to the cell suspension 30 min before irradiation.

The aligots containing cells, which were placed in a 37°C water bath, were irradiated using a ^{60}Co gamma radiation unit with a dose rate of 0.76 Gy/min.

After drug and/or radiation treatment, the cells were rinsed twice with MEM to remove the drugs from the medium, inoculated at a number to yield 100–500 colonies per tube, and incubated for 14 days to allow colony formation.

Their plating efficiency was higher than 90%. The surviving fraction was determined as the ratio of colonies counted to cells seeded, considering the plating efficiency of control cells. The five experiments were performed independently.

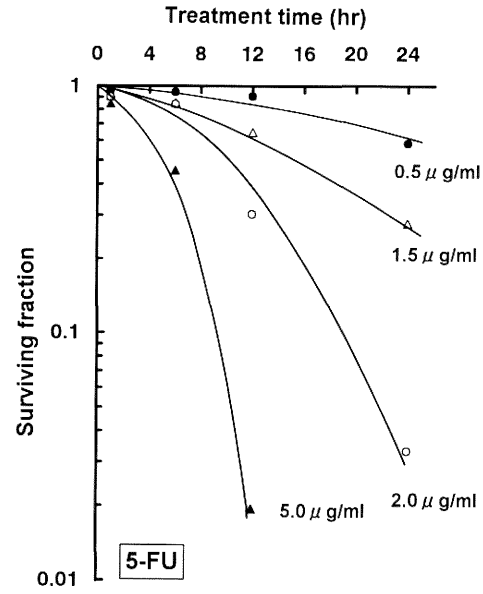

Fig. 1. Effects of 5-FU on survival of FM3A cells treated with various doses of 5-FU up to 24 h.

RESULTS

Initially the effects of the drugs alone on FM3A cell survival were investigated. Fig. 1 shows the effects of continuous exposure to different concentrations of 5-FU up to 24 h. The survival fraction of cells was progressively reduced as the duration of exposure to the drug increased. Thus, as exposure time increased, significantly less of the drug was required to achieve any given level of cell killing. Meanwhile, results of continuous exposure to CDDP are seen in Fig. 2. Also noted is the progressive increase in cell killing with the longer duration of exposure.

The effects of the continuous exposure of CDDP simultaneously combined with radiation were compared with those of pulse exposure. A dose of 1.0 μg/ml for 1-hour exposure and 0.1 μg/ml for 24-hour exposure of CDDP were chosen, because they showed similar levels of cell killing by the drug alone. As shown in Fig. 3, radiation plus continuous exposure to CDDP resulted in a mean lethal dose (D_0) value which was a little less than that produced by radiation alone and radiation plus pulse exposure to CDDP.

From the results in Figs. 1 and 2, a dosage of 0.5 μg/ml of 5-FU and 0.1 μg/ml of CDDP was chosen for the experiments of concurrent continuous exposure of the drugs in combination with radiation. Both of the drug concentrations are considered to show relatively low toxicity by themselves and to be applicable clinically.[11,12] In these experiments, a graded

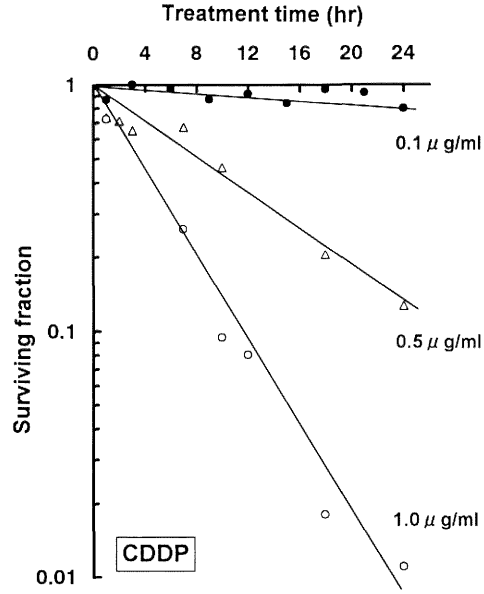

Fig. 2. Cell survival curves of FM3A cells treated with various doses of CDDP up to 24 h.

dose of radiation was given at 30 min after the initiation of continuous drug exposure. Survival curves were constructed in Fig. 4, containing that of radiation alone and radiation plus 5-FU CDDP, and both drugs. By adding either CDDP or 5-FU to the radia-

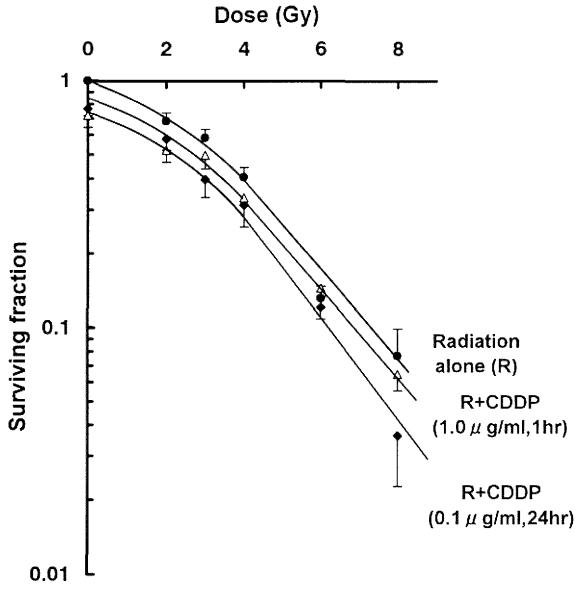

Fig. 3. Cell survival curve of FM3A cells treated with radiation alone (●); radiation delivered in the middle of 1-hour pulse exposure to 1.0 μg/ml CDDP (△); radiation delivered at 30 min after the initiation of 24-hour continuous exposure to 0.1 μg/ml CDDP (◆).

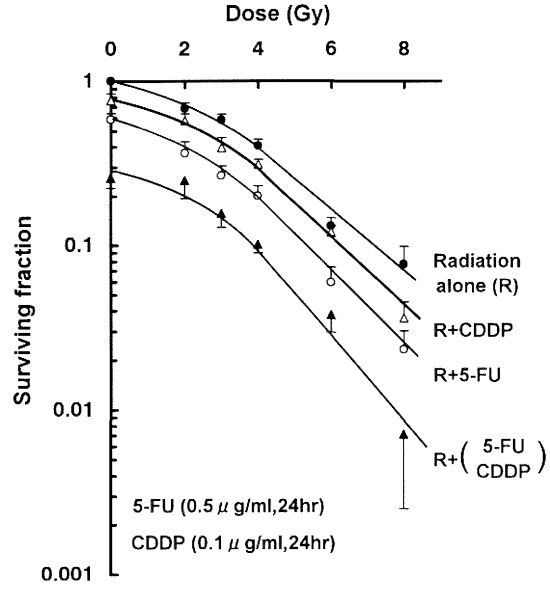

Fig. 4. Cell survival curve of FM3A cells treated with radiation alone (●); radiation delivered at 30 min after the initiation of 24-hour continuous exposure to 0.5 μg/ml 5-FU (○), 0.1 μg/ml CDDP (△), and 0.5 μg/ml 5-FU plus 0.1 μg/ml CDDP (▲).

Table 1. D_0 and enhancement ratios (ER) in the concurrent treatment of either 5-FU, CDDP, or both combined with radiation

	D_0	ER
RT alone	2.2	—
RT+5−FU (0.5 μg/ml, 24 h)	1.9*	1.16
RT+CDDP (1.0 μg/ml, 1 h)	2.2	1.00
RT+CDDP (0.1 μg/ml, 24 h)	2.1	1.05
RT+5−FU (0.5 μg/ml, 24 h) +CDDP (0.1 μg/ml, 24 h)	1.7*	1.30

* $p < 0.005$

tion, the slope of the survival curve changed and the D_0 decreased. In cases where both drugs were added, a slightly higher decrease in D_0 was observed (Table 1).

DISCUSSION

Continuous administration of 5-FU or CDDP can be employed in order to improve the therapeutic index, either by increasing the antitumor effect or by reducing systemic toxicity; in some cases this can be achieved simultaneously. 5-FU, one of the S phase specific agents, has a short plasma half-life and is well suited for long-term continuous administration which increases the fraction of cells exposed during the S phase of the cell cycle. Meanwhile, CDDP is considered to be non-specific for the cell cycle phase. However, pharmacokinetic advantages for the continuous administration of CDDP have been suggested.[12,13] The active platinum compound is non-protein-bound or filtrable platinum. It is likely that the reduced peak level of filtrable platinum obtained in continuous infusion may result in a smaller degree of toxicity, while efficacy might be related to the increased concentration-time product of filtrable plasma platinum levels. Preclinical studies have suggested that continuous low dose exposure to CDDP results in killing cells more effectively when compared to pulsed high dose exposure.[14,15]

Another approach is to exploit the increased response of radiation by combining this simultaneously with cytotoxic drugs. Preclinical studies have underscored the importance of time-dose relationships for the optimal administration of drugs and radiation. *In vitro* studies on the effect of 5-FU in conjunction with radiation have demonstrated that radiosensitization by 5-FU is a post-irradiation phenomenon, and that the exposure of cells to 5-FU prior to radiation has no sensitizing effect, this being maximized when 5-FU is

present for extended durations beyond the cell doubling time after radiation exposure.[6,7]

For CDDP combined with radiation, a radiosensitizing effect resulting in enhanced cell killing not only under aerobic but also hypoxic conditions has been demonstrated in laboratory studies.[8-10] In the present study the effects in the hypoxic condition have not been considered, since all experiments have been done under normoxic conditions, *In vitro* studies have demonstrated that CDDP modifies radiation dose response curves for cultured mammalian cells.[9,10] However, little is known about the effect of the cotinuous exposure of CDDP in combination with radiation. In the present study, pulse exposure of CDDP did not alter D_0 value, while radiosensitization, defined in terms of decreases in D_0, was obtained by continuous exposure (Fig. 3). This indicates that in this experimental system the degree of radiosensitization does not appear to be drug dose dependent and radiosensitization may require lengthy drug exposure following irradiation.

The interactions between 5-FU plus CDDP and radiation appear to be complex. Concurrent continuous exposure of 5-FU and CDDP combined with radiation resulted in a slightly higher decrease in D_0, that is, a greater radiosensitizing effect when compared with the regimen combining 5-FU or CDDP with radiation (Fig. 4). It is likely that CDDP inhibits potentially lethal radiation damage repair (PLDR) and also sublethal radiation damage repair (SLDR),[10,16] while 5-FU inhibits PLDR[17] but has no effect on SLDR.[6] Although the underlying mechanisms of radiosensitization are at present poorly understood, inhibition of radiation damage repair may play a role in enhanced cell killing. Therefore, it is possible that this drug combination inhibits PLDR synchronously, which can lead to a greater radiosensitizing effect. Moreover, CDDP has the effect of potentiating the antitumor effectiveness of 5-FU, which is known as biochemical modulation involving the pharmacologic manupulation of the intracellular pathway of 5-FU and resulting in the increased inhibition of DNA synthesis.[18-20] The effectiveness of the concurrent administration of the two drugs may be explained in part by this pharmacologic advantage.

These data showing that concurrent continuous exposure of 5-FU and CDDP potentiates radiosensitization support the concept that this type of concurrent chemoradiotherapy is a useful approach to improve the local control of radioresistant tumors. However, it should be borne in mind that normal tissues in the irradiated volume also show enhanced responses. When the enhanced toxicity in normal tissues is tolerable, clinical trials should be designed to evaluate the therapeutic advantages of this regimen.

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