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In vivo comparative therapeutic study of optimal administration of 5-fluorouracil and cisplatin using a newly established HST-1 human squamous-carcinoma cell line*

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Summary. The efficacy and toxicity of 5-fluorouracil (5-FU) and cisplatin (CDDP) given in a sequential combination were evaluated in nude mice transplanted with HST-1, a newly established human squamous-carcinoma cell line. 5-FU and CDDP were given i.p. for 5 days and 1 day, respectively, either as single agents or in a sequential manner separated by a 24-h interval. The treatment was repeated every 30 days. Although inhibition of tumor growth was seen in all of the treated groups after two cycles, the sequence of 5-FU followed by CDDP significantly reduced the tumor burdens throughout all three courses and was more effective than the reverse sequence or either drug alone. Neither treatment-related death nor significant hematologic or nephrologic toxicities were seen, even following three cycles of therapy. Significant weight loss was observed only in mice treated with CDDP followed by 5-FU. This sequence dependence of the activity and toxicity of the 5-FU and CDDP combination should thus be incorporated into the design of a clinical trial.

Introduction

The combination of cisplatin (CDDP) and 5-fluorouracil (5-FU) has been shown to display synergistic antitumor activity in several preclinical studies [13, 15, 16]. This combination has shown marked therapeutic efficacy in human malignancies that are relatively refractory to chemotherapy, such as head and neck cancer [1, 3] and, more recently, colorectal carcinoma [6]. However, the optimal order of administration of these drugs in combination therapy has yet to be determined. The sequence of CDDP followed by 5-FU has been shown to be more cytotoxic than the reverse succession in in vitro studies [15], whereas the sequence of 5-FU followed by CDDP has proved to be more active than the opposite order of administration in tumor-bearing animals [13]. The difference in these in vivo and in vitro results prompted us to study the optimal administration schedule for these drugs in human tumor xenografts in nude mice, since the data derived from treatment of human tumor xenografts in nude mice have been shown to correlate well with the results of clinical trials [5]. The present report describes our evaluation of the efficacy of different sequences of 5-FU and CDDP combinations in human tumor xenografts.

Materials and methods

Drugs. 5-FU and CDDP were kindly provided by Kyowa Hakko Co., Tokyo, and Bristol-Myers Co., Japan, respectively. Immediately before their use, 5-FU was dissolved in distilled water and CDDP was dissolved in sterile physiological saline. Drugs were given i.p. in a volume of 0.2 ml.

Cell line. The highly tumorigenic human squamous-carcinoma cell line HST-1 [10] was cultured in Dulbecco's modified minimum essential medium (Gibco, Grand Island, N. Y.) supplemented with 10% fetal calf serum (Gibco). Cell cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37° C. For heterotransplantation, approximately 1×10^7 tumor cells were inoculated s.c. into the right flank of the nude mice.

Animals. BALB/c nu/nu male mice (6 weeks old) were obtained from the Japan Crea Co. They were maintained under aseptic conditions that included filtered air and sterilized food, water, bedding, and cages.

In vivo evaluation of therapeutic effectiveness and toxicity. After confirmation of tumor growth, nude mice bearing HST-1 tumors were divided into five groups of five to six animals according to the following treatment schedules: (a) 4 mg/kg CDDP given i.p. on day 1, (b) 24 mg/kg 5-FU injected i.p. on days 1–5, (c) 4 mg/kg CDDP given i.p. on day 1 and 24 mg/kg 5-FU injected i.p. on days 2–6, (d) 24 mg/kg 5-FU given i.p. on days 1–5 and 4 mg/kg CDDP injected i.p. on day 6. In the controls, equal volumes of physiological saline were injected i.p. on days 1–6. The treatments were repeated every 4 weeks for three courses.

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Table 1. Therapeutic efficacy of various treatments

Compound	Dose (mg/kg)	Mice (*n*)	Tumor volume (mm^3)		
			Day 36	Day 63	Day 70
CDDP	4	5	188 ± 46.3	405.1 ± 192.1	459.4 ± 262.1
5FU	24	5	143.2 ± 61.8	310.2 ± 113.1	403 ± 139.4
CDDP→5FU	4→24	6	190.5 ± 61.6	356.7 ± 157.3	527 ± 377.7
5FU→CDDP	24→ 4	6	79 ± 36.9*	192.8 ± 85.4**	163.3 ± 79.1**
Controls	–	5	386.3 ± 130.6	932.9 ± 253.7	991 ± 266

Data represent mean values ± SE
* $P < 0.05$, ** $P < 0.01$ vs control value

Table 2. Toxicity of 5FU and CDDP chemotherapy

Compound	Dose (mg/kg)	Mice (*n*)	WBC ($\times 10^4/mm^3$) Day 68	Hb (g/dl) Day 68	Platelets ($\times 10^4/mm^3$) Day 68	Creatinine (mg/dl) Day 77
CDDP	4	5	7,775 ± 910.5	13.9 ± 0.95	54.4 ± 5.98	0.6 ± 0.13
5FU	24	5	8,380 ± 1,346.6	14 ± 0.61	71.9 ± 5.02	0.65 ± 0.07
CDDP→5FU	4→24	6	8,016.7 ± 868.1	12.8 ± 0.57	62.5 ± 10.9	0.52 ± 0.05
5FU→CDDP	24→ 4	6	9,533.8 ± 415.3	13.1 ± 1.02	77.2 ± 11.5	0.42 ± 0.04
Controls	–	5	10,500 ± 1,284.1	14.2 ± 0.44	66.4 ± 12.6	0.45 ± 0.03

Data represent mean values ± SE

Table 3. Change in body weight after chemotherapy

Compound	Dose (mg/kg)	Mice (*n*)	Body weight change (%)			
			Day 7	Day 36	Day 63	
CDDP	4	5	108.9 ± 0.16	117 ± 1.69	112.5 ± 1.69	
5FU	24	5	110.8 ± 2.27	116.2 ± 1.76	115.7 ± 2.81	
CDDP→5FU	4→24	6	98.6 ± 3.29***	92.3 ± 2.2***	89 ± 2.59**	[a]
5FU→CDDP	24→ 4	6	99.6 ± 2.02*	100.1 ± 3**	97.2 ± 2.19*	[a]
Controls	–	5	112.5 ± 2	120.9 ± 3.2	110 ± 3.86	

Data represent mean values ± SE
* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ vs control value
[a] $P < 0.05$

For evaluations of therapeutic efficacy, tumor volumes were measured weekly and were calculated using the following formula:

$$V = A \times B^2/2,$$

where *A* represents the largest diameter and *B*, the smallest diameter. Toxicity was assessed by the determination of changes in body weight, WBC, hemoglobin levels, platelet count, and serum creatinine values. Animal body weights were recorded immediately before the initiation of treatment and once weekly thereafter. The change in body weight was calculated as a percentage of the initial body weight. WBCs, hemoglobin values, and platelet counts were measured 10 days after the initiation of the last therapy. Serum creatinine levels were calculated at 3 weeks following the last treatment. For determination of the complete blood count, blood was collected from the orbital sinus and assessed electronically. Serum creatinine was assayed using a Hitachi 705 autoanalyzer.

Statistical analysis. Student's *t*-test was used for statistical analysis of the data.

Results

Table 1 shows the effect of the therapy on changes in tumor volumes. As single agents, 5-FU and CDDP slightly reduced the tumor size. This efficacy became particularly evident after two cycles of treatment. However, the administration of these two drugs as single agents alone was inadequate to suppress the growth of HST-1 cells for 3 months. When the same doses (24 mg/kg 5-FU, 4 mg/kg CDDP) were combined in a sequential manner, the sequence of CDDP followed by 5-FU failed to produce either a synergistic or even an additive therapeutic effect, showing a tumor inhibition comparable with that obtained by treatment with 5-FU or CDDP alone. In contrast, when the same doses were given in the opposite sequence, statistically significant reductions in tumor growth were achieved throughout the entire course of treatment. The sequence of 5-FU followed by CDDP appeared to be more active than the reverse sequence, although the difference was not statistically significant.

During the three courses of the different treatments, there was no instance of either toxic or spontaneous death, and neither hematologic nor nephrologic toxicities were noted (Table 2). Table 3 shows the body weight of the animals, reflecting the overall toxicity of the treatment. No significant weight reduction was caused by treatment with either 5-FU or CDDP alone after three courses of therapy. However, animals treated with the combination therapy lost a substantial amount of weight. The loss of body weight became much more obvious as the combination therapy was repeated, indicating the occurrence of chronic toxicity. On day 67 (7 days after the last treatment), the reduction in body weight in mice treated with the sequence of CDDP followed by 5-FU was more significant than that in mice treated with the opposite sequence ($P < 0.05$). Moreover, progressive weight losses were found only in mice treated with CDDP followed by 5-FU (Table 3).

Discussion

Using the HST-1 human tumor xenotransplanted into nude mice, we demonstrated that the sequential combination of 5-FU followed by CDDP is more effective than the reverse sequence or either drug alone. This combination has been shown to produce synergistic cytotoxicity in in vitro studies [15] and in tumor-bearing animals [13, 16]. Scanlon et al. [15] have shown that CDDP followed by 5-FU is more cytotoxic than the reverse sequence. Since thymidine reversed the interaction, these authors ascribed this enhancement of antitumor activity to the CDDP-induced increase in intracellular levels of reduced folates, which potentiate the action of 5-fluorodeoxyuridine monophosphate by forming a covalent ternary complex with thymidylate synthase [2]. In contrast, an in vivo study using a primary tumor model in mice has demonstrated that the sequence of 5-FU followed by CDDP is more active than the opposite sequence [13]. The present data support the latter finding.

The biochemical mechanism for this interaction remains unclear. The repair of CDDP-induced DNA damage has been shown to be inhibited by the antimetabolites for DNA synthesis, resulting in a potentiation of cytotoxicity [17]. Moreover, the enhanced repair of these lesions correlates with resistance to CDDP [4, 9]. Since 5-FU has been shown to induce DNA lesions indirectly by DNA-repair inhibition via an unknown mechanism that does not involve the incorporation of the drug into DNA [7, 8], pretreatment with 5-FU may cause an inhibition of the CDDP-induced DNA-adduct repair. Also, evidence has been presented that a misincorporation of 5-FU into RNA might be associated with a block in processing and/or nuclear cytoplasmic transport, thus producing cytotoxicity by interference with the maturation of nuclear RNA [12]. These alterations may result in the depletion of important enzymes for the intoxication of CDDP and/or CDDP-induced DNA damage repair.

The toxicity of the combination of 5-FU and CDDP appears to be sequence-dependent. Although no difference in hematologic or nephrologic toxicity was noted among these sequential treatments, the weight losses that reflect the overall toxicity were much more evident for the sequence of CDDP followed by 5-FU despite its lower antitumor activity. Pratesi et al. [13] have reported similar results. Although the mechanims underlying this reduced toxicity in the presence of higher antitumor activity remains unclear, it might reflect the sequence-dependent differences in repair kinetics between the tumor cells and the cells in normal tissue.

Although the clinical efficacy of this drug combination has been confirmed [1, 3, 6, 14], the sequence-dependent antitumor activity previously demonstrated in animals has yet to be clinically recognized. Based on the non-cross-resistance, the nonoverlapping toxic effects, and the synergistic mechanisms of action, we have launched a phase I/II trial of this sequential combination in the treatment of non-small-cell lung cancer and have found that it is highly effective against this disease and produces minimal toxicity, yielding an overall response rate of nearly 80% [11]. Although this trial was preliminary, this agreement may justify our preclinical study and support further study of the optimal administration of anticancer drugs.

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