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Sequence dependence of the antitumor and toxic effects of 5-fluorouracil and *cis*-diamminedichloroplatinum combination on primary colon tumors in mice*

G. Pratesi[1], L. Gianni[2], C. Manzotti[1], and F. Zunino[1]

Divisions of [1] Experimental Oncology B and [2] Medical Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian, 1, I-20133 Milan, Italy

Summary. Primary colon tumors of different sizes and malignancy, chemically induced by methylazoxymethanol in outbred CF-1 mice, were used to investigate the antitumor effects of 5-Fluorouracil (5FU) and *cis*-diamminedichloroplatinum (DDP), given weekly i.v. as single agents or in combination. When single-drug chemotherapy was tested, DDP showed higher efficacy than 5FU. In fact, in two separate experiments a significant reduction ($P < 0.05$) of tumor number (TN) and tumor burden was obtained by treatment with the optimal dose of DDP (4.5 mg/kg per injection) and not by that of 5FU (52 mg/kg). When the two drugs were combined (24-h interval), studies carried out on healthy mice treated weekly i.v. showed a lower toxicity with the same doses given in the sequence 5FU-DDP than in the opposite sequence. The two drugs, delivered in the sequence 5FU followed by DDP, statistically reduced the TN and total tumor burden compared to control mice ($P < 0.05$). On the other hand, the same doses in the sequence DDP followed by 5FU did not attain significant tumor reduction. The sequence dependence of the activity and toxicity of the 5FU and DDP combination observed in this experimental model should be taken into account in the design of clinical trials.

Introduction

Human gastrointestinal cancers are usually refractory or poorly sensitive to chemotherapy. The challenging task of finding new drugs or drug combinations for these malignancies is further complicated by the lack of good, predictive experimental models to test new therapeutic strategies that could be transferred to the human situation.

In 1977 Schabel et al. [21] underlined the differences between experimentally transplanted and naturally occurring tumors in man as possible reason for the lack of positive correlation sometimes observed in tumor chemosensitivity [25]. Pharmacokinetic and metabolic differences between mice and humans can further complicate the goal of transferring experimental observations to the clinical setting.

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Abbreviations used: MAM, methylazoxymethanol acetate; 5FU, 5Fluorouracil; DDP, cis-diamminedichloroplatinum; TN, tumor number; TTB, total tumor burden; Ara-C, cytosine-arabinoside

Offprint requests to: Dr. G. Pratesi

Recently we have shown that primary colon tumors chemically induced by MAM[3] in CF-1 female mice may represent an useful experimental model which better mimics the clinical situation than models employing transplantable tumors [18]. In the case of MAM-induced tumors, outbred mice with neoplasia of different size and malignancy can be treated, and the tumors have histologic and growth features that more closely resemble those of human colon carcinoma [8, 23, 27]. Thus, in this work the MAM model was used to evaluate the toxicity and efficacy of a two-drug regimen that combines 5FU and DDP. This combination has shown definite activity in human malignancies that are relatively refractory to chemotherapy, such as squamous cell carcinomas of esophagus [12, 15] and head and neck [1, 7, 19].

Since in experimental models different sequences of 5FU and DDP yield different results [24, 26], in this study the effects of 5FU and DDP combination were compared according to opposite sequences, using a weekly treatment schedule and 24-h interval between the two drug administration.

Materials and methods

Primary induced colon tumors. The detailed description of the experimental model has previously been reported [18]. Briefly, subcutaneous (s.c.) injections of MAM (0.4 mg/mouse) were administered once a week for 10 weeks to outbred CF-1 female mice. The animals were then treated with the drugs starting 21–23 weeks from the first MAM injection, because at this time all the mice should bear measurable tumors on the colonic mucosa. Mice were sacrificed one week after the last chemotherapy treatment and each large intestine was fixed and macroscopically inspected. Tumor Number (TN) along the colonic mucosa was assessed and three diameters of each tumor were measured by vernier caliper. Single tumor volumes resulting from the product of the three diameters and expressed in mm^3 were summed to give Total Tumor Burden (TTB) for each mouse. The average values of TN and TTB for each group were evaluated only for mice surviving until the end of the experiments (27th or 28th week). Mice "spontaneously" dead before this time were not evaluated, because death may have been due to many unrecognizable factors, such as tumor burden, intestinal occlusion or drug toxicity. Lethal toxicity of the drug treatment was assessed in healthy female CF-1 mice treated in parallel.

The results of the chemotherapy studies are reported in each table as a single experiment because of a large variability among the experiments in the TN and TTB of the control mice, due to the fact that we dealt with induced and not transplantable tumors.

Antitumor drugs. 5FU (clinical preparation by Roche S. p. A., Milan, Italy) was dissolved in distilled water; DDP, kindly provided by Bristol-Myers Co. (Wallingford, CT, USA), was dissolved in saline. Drugs were administered i.v. in a volume of 10 ml/kg of body weight.

Statistics. The Mann-Whitney rank test was used for statistical comparison of tumor-bearing mice. Chi-square with correction for continuity was used for toxicity studies.

Results

Since 5FU activity in clinical trials has been reported to be dependent on treatment schedule [17], a first experiment was designed to compare the activity of the drug delivered i.v. according to two different schedules: a) once a week for 7 weeks according to a previous study [18]; b) once a day for 3 consecutive days for weeks 21, 24 and 27. For both schedules the treatment started 21 weeks after the first MAM injection and the mice were sacrificed at the 28th week. Table 1 shows that 5FU given according to either schedule only slightly reduced TN and TTB, except for the optimal dose of the weekly schedule (46 mg/kg). In the latter case, a borderline statistically significant reduction ($P < 0.1$) of TTB was reached. Comparison with DDP activity was therefore carried out according to the weekly treatment schedule.

Table 2 shows the results of the toxicity studies, on healthy CF-1 female mice, treated with 5FU and DDP, single or combined, delivered i.v. according to a weekly schedule for 4–6 times. Maximum tolerated doses (i.e., around LD10) were 52 and 4.5 mg/kg for 5FU and DDP, respectively. In combination studies, fractions equivalent to 1/3, 2/3 and full LD10 dosages of each drug were combined to determine the toxicity when both drugs were delivered i.v. with a 24-h interval. A striking observation was that, when the two drugs were delivered according to the sequence DDP-5FU (DDP 3 mg/kg → 5FU 35 mg/kg), a significantly higher number of toxic deaths (9/15) was observed as compared to the same dose levels in the opposite sequence (2/24; $P < 0.01$).

Tables 3 and 4 show the effects, on primary colon tumors, of 5FU and DDP alone or in combination at selected doses. From the presented data it can be seen that the tumors showed low sensitivity to 5FU which attained a significant reduction of TN and TTB only in one experiment (Table 4). In contrast, the optimal dose of DDP (4.5 mg/kg) significantly affected both parameters in two experiments ($P < 0.05$ or lower).

When inactive doses of the two drugs were combined, i.e. 5FU 35 mg/kg and DDP 3 mg/kg (Table 3), a therapeutic benefit could be achieved, being the tumor inhibition comparable to that obtained by the optimal DDP dose. The data presented in Table 4 confirm this result. Moreover when these same doses were given in the opposite sequence, i.e. DDP → 5FU (Table 4), lower and insignificant therapeutic effect was achieved. In addition this sequence resulted more toxic, because 5 mice of this group died before the end of the experiment as compared to only 1 in the group treated with the sequence 5FU → DDP.

Table 2. Toxicity of 5FU and DDP given weekly i.v., single or combined, on healthy CF-1 female mice

No. of treatments	Compound	Dose mg/kg	Dead mice/ total mice[a]	Mortality %
–	Controls	–	0/20	
6	5FU	35	0/10	
6	5FU	52	1/16	6
5	5FU	70	3/10	30
6	DDP	3	0/10	
6	DDP	4.5	2/17	12
4	DDP	6	8/10	80
6	5FU → DDP	17.5+3	0/10	
5	5FU → DDP	17.5+4.5	3/9	33
6	5FU → DDP	35+1.5	1/9	11
6	5FU → DDP	35+3	2/24	8
6	5FU → DDP	52+1.5	3/9	33
6	DDP → 5FU	3+35	9/15	60[b]

[a] 1 week after last drug treatment
[b] Significantly different from the sequence 5FU + DDP, using the same dose levels. $P < 0.01$ by chi-square test

Discussion

The data obtained in this study on the antitumor activity of 5FU and DDP given as single agents indicate a higher activity of the latter on both TN and TTB. In fact, 5FU at its optimal dose attained a marginal effect (significant reduction of either TN or TTB in 1 out of 3 different experiments). In contrast, the optimal dose of DDP significantly reduced TTB and TN in all the experiments.

Table 1. Activity of 5FU given i.v. according to two treatment schedules starting 21 weeks after the first MAM injection

Treatment schedule	Dose mg/kg	No. of mice[a] evaluated/total	Tumor growth at 28th week	
			Average TN ± SE	Average TTB ± SE (mm^3)
Controls		10/19	9.8±1.3	269.9±46.4
Every 7 days × 7	46	11/15	8.6±1.1[b]	162.7±29.7[c]
	70	5/15	11.2±3.5[b]	186.9±54.3[b]
Every day × 3	20	10/13	8.8±1.6[b]	213.4±64.0[b]
Weeks 21, 24, 27	30	4/15	13.2±3.7[b]	207.8±28.7[b]

[a] The mice not included in the evaluation died spontaneously
[b] Not significant
[c] $P < 0.1$ by Mann-Whitney rank test

Table 3. Chemotherapy treatment (i.v. weekly × 5) starting 23 weeks after the first MAM injection

Compound	Dose mg/kg	No. of mice[a] evaluated/total	Tumor growth at 28th week	
			Average TN ± SE	Average TTB ± SE (mm^3)
Controls	–	19/20 (1)	15.9 ± 2.2	343.6 ± 49.2
5FU	35	19/20 (3)	11.6 ± 1.9[b]	258.0 ± 40.9[b]
5FU	52	11/20 (1)	10.6 ± 2.3[b]	243.1 ± 65.9[b]
DDP	3	15/20 (2)	10.3 ± 2.2[b]	251.2 ± 73.7[b]
DDP	4.5	17/20 (0)	9.8 ± 1.6[c]	179.7 ± 28.7[c]
5FU → DDP	17.5 + 3	17/19 (1)	10.5 ± 1.8[b]	269.3 ± 65.0[b]
5FU → DDP	35 + 1.5	18/20 (0)	10.4 ± 1.4[b]	164.6 ± 29.5[c]
5FU → DDP	35 + 3	17/20 (1)	8.2 ± 1.8[c]	170.0 ± 45.7[c]

[a] Mice not included died spontaneously. Mice without tumors are reported in parenthesis and were included
[b] Not significant
[c] $P < 0.05$ by Mann-Whitney rank test

Table 4. Chemotherapy treatment (i.v. weekly × 6) starting 23 weeks after the first MAM injection

Compound	Dose mg/kg/inj	No. of mice[a] evaluated/total	Tumor growth at 29 weeks	
			Average TN ± SE	Average TTB ± SE (mm^3)
Controls	–	22/22 (0)	7.8 ± 0.9	268 ± 45
5FU	52	22/23 (4)	4.3 ± 0.8[d]	145 ± 30[c]
DDP	4.5	21/24 (4)	4.4 ± 0.8[d]	76 ± 13[d]
5FU → DDP	35 + 1.5	23/23 (1)	5.6 ± 1.1[c]	163 ± 29[c]
5FU → DDP	35 + 3	23/24 (5)	3.8 ± 0.9[d]	91 ± 19[d]
DDP → 5FU	1.5 + 35	23/24 (1)	5.9 ± 1.2[e]	161 ± 36[e]
DDP → 5FU	3 + 35	18/23 (2)	5.9 ± 1.2[b]	150 ± 39[e]

[a] Mice not included died spontaneously. Mice without tumors are reported in parenthesis and were included
[b] Not significant by Mann-Whitney rank test;
[c] $P < 0.05$; [d] $P < 0.01$; [e] $P < 0.1$

DDP is also known to be active in experimental murine transplantable tumors of the large bowel [28]. Clinical studies failed to demonstrate activity for DDP in heavily pretreated patients with metastatic colon carcinomas [5, 13]. A minimal activity was found in untreated advanced colorectal cancer patients [9]. However, in 30 patients with metastatic or recurrent colon cancer, 18 partial and 3 complete responses were obtained with intra-arterial administration of DDP [14]. It may be that the intra-arterial route allows tumor cell exposure to higher drug levels than the i.v. route. The efficacy of DDP therapy in murine models might reflect the higher plasmatic drug level achievable in mouse as compared to man [3].

In our study the combination of 5FU (35 mg/kg) followed 24 after by DDP (3 mg/kg) significantly reduced both TN and TTB, which were not affected by the same doses of the single drugs. The antitumor effects of this combination were similar to those obtained by DDP alone at its optimal dose of 4.5 mg/kg. This observation may have clinical implications, since the clinical use of high dose DDP is limited by renal and neurological toxicity, and the possibility of obtaining the same therapeutic effect using lower DDP doses might be of value.

The biological and biochemical bases for the observed potentiating effects between 5FU and DDP remain to be elucidated. The activity of DDP is also potentiated by other antimetabolites such as Ara-C [2] and methotrexate [22]. The hypothesis has been put forward that the mechanism of synergism is related to interference of the antimetabolite with repair of DDP-induced DNA damage, since combined treatment increases cross-link formation [2].

A DDP-induced increase of intracellular level of reduced folates, a cofactor in the formation of the complex of FdUMP and thymidylate synthase, has been recently reported as additional biochemical basis for DDP and 5FU synergism [20].

The sequence-dependent toxicity of the combination 5FU-DDP could be also due to alterations of the physiological cell metabolism, including depletion of hepatic reduced glutathione, produced by DDP treatment [16]. In fact it has been reported that sulfhydryl compounds critically influence the toxicity of 5FU in mouse [6].

On the basis of the proposed biochemical mechanisms, one would expect that a potentiation of the antitumor activity is associated to a proportional potentiation of the toxic effects. However, in the present study an unexpected superior activity was observed using the less toxic sequence, i.e. 5FU → DDP, and it is evident that other, more selective mechanisms must be involved in the therapeutic synergism of this drug combination.

In conclusion, our study shows an enhancement of DDP activity by combination with 5FU against murine primary colon tumors closely resembling the human counterpart. Inherent metabolic and pharmacokinetic differences between a mouse model and humans prevent the direct transfer of our observations to a clinical setting. How-

ever, the efficacy of the combination is in agreement with the results of some clinical trials [4, 10, 11], and the observed influence of drug sequence in the combination of 5FU and DDP may have an as yet unrecognized correspondance in humans, which should be taken into account in designing future trials.

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