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Continuous infusion of 5-fluorouracil plus low-dose cisplatin in tumor-bearing mice

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Combination chemotherapy with 5-fluorouracil (5-FU) and cisplatin is widely used but the optimal daily dose of the latter has not been established. The present study was designed to determine the pharmacodynamic interaction and antitumor effect of these drugs when used at clinically relevant doses in animals. Male ddY mice were injected with Sarcoma 180 cells and then given a continuous infusion of 5-FU (10 mg/kg/day) for 5 days (via an implanted micropump) ± cisplatin 1 mg/kg by i.p. bolus (pharmacodynamic study). Alternatively, mice were given 5-FU ± cisplatin (0.2 mg/kg/day), cisplatin alone for 5 days or no treatment (anti-tumor effect study). Tissue and serum samples were taken for analysis. No significant differences in serum pharmocokinetics were observed between the groups at any timepoint but 0.5 h after the start of infusion, where the serum 5-FU concentration was lower with monotherapy than with combination therapy. 5-FU alone had a slight antitumor effect, while low-dose cisplatin alone had no noticeable effect. 5-FU + cisplatin produced a favorable response, with a significantly lower treated/control tumor weight ratio than in the non-treated and cisplatin monotherapy groups ($p < 0.05$), although this was not significantly different from the response to 5-FU monotherapy. In conclusion, the dose of cisplatin used in the present study was insufficient to generate a significant tumor response alone but it did tend to enhance the antitumor efficacy of 5-FU. Continuous 5-FU infusion plus low-dose daily cisplatin may become clinically useful after further investigation.

Key words: Treated:control ratio of tumor weights, tetrahydrofolate, thymidylate synthase, white blood cells.

Introduction

Combination therapy with 5-fluorouracil (5-FU) and cisplatin has been widely used since it was suggested that the antitumor effect of 5-FU may undergo biochemical modulation by cisplatin. An *in vitro* study by Scanlon *et al.* first provided the biochemical basis for the synergistic antitumor effect of 5-FU combined with cisplatin[1] that was established *in vivo* by Shirasaka *et al.*[2,3] Clinical application of 5-FU and cisplatin combination chemotherapy was attempted by Loehrer *et al.*[4] and Bernal *et al.*,[5] who obtained favorable responses in colorectal and head/neck cancer in several patients receiving 5-FU and standard doses of cisplatin for the respective malignancies. In both studies, combination therapy caused severe toxicity, including treatment-related deaths. A favorable response in colorectal cancer has also been obtained with 5-FU infusion plus low ($20\ mg/m^2$) doses of cisplatin, although the regimen also caused gastrointestinal or marrow toxicity more frequently than 5-FU monotherapy[6] and death from sepsis in one patient.[7] Under this regimen, adequate hydration successfully prevented the renal toxicity of cisplatin.[6,7] More recently, 5-FU plus dialy cisplatin given at an even lower dose ($6–10\ mg/m^2$) has achieved a good response in gastric and colorectal cancers.[8–11] Since 5-FU infusion plus low-dose daily cisplatin caused only mild toxicity, with no reports of renal toxicity, and did not require hydration to prevent the adverse effects of cisplatin, this regimen may gain wide acceptance if its efficacy is established.

No consensus has been reached on the optimal daily dose of cisplatin combined with 5-FU and only a few experimental studies have addressed this combination therapy. The present study was performed using an animal model of continuous 5-FU infusion to determine the pharmacodynamic interaction between 5-FU and cisplatin given at a dose equivalent to that used clinically for this combination.

Materials and methods

Animals and tumor cells

Six-week-old male ddY mice weighing 30 g were used. Sarcoma 180 grown in mice was selected because the cell line has confirmed susceptibility to both 5-FU and cisplatin. Each mouse received

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5×10^6 tumor cells s.c. into the lateral region of the abdomen.

Pharmacodynamic study

Two groups of 15 tumor-bearing mice were used. Eight days after tumor transplantation each mouse was implanted i.p. with an Alzet micropump (model 1007D, 15 mm long, 6 mm diameter; Alza, California, USA) through a skin incision made on the abdomen under ether anesthesia. The pump was prefilled with a 5 day supply of 5-FU solution (10 mg/kg/day × 5 in 60 μl) and designed to deliver solution continuously at 0.5 μl/h after implantation. Mice randomized to 5-FU plus low-dose cisplatin were given 1 mg/kg of cisplatin as a bolus i.p. injection immediately after skin closure.

Three mice from each group were sacrificed to obtain serum and tumor tissue specimens at each of the following times after dosing: 0.5, 1, 3, 6 and 12 h. Specimens were stored at −80°C until HPLC assay for 5-FU. Specimens obtained from three mice at one time were combined and then subjected to HPLC assay. All assays were performed in duplicate. A serum or tissue 5-FU concentration–time curve was determined and the area under the curve (AUC) was calculated using the trapezoidal rule.

Antitumor-effect study

This study utilized four treatment groups of seven tumor-bearing mice: (i) continuous 5-FU infusion (10 mg/kg/day) via an i.p. implanted micropump, (ii) daily i.p. doses of cisplatin (0.2 mg/kg/day), (iii) continuous 5-FU infusion plus daily and (iv) untreated controls. The daily doses of 5-FU and cisplatin were equivalent to those used clinically for combination chemotherapy in our department. Treatment was initiated 3 days after tumor transplantation and continued for 5 days. Eight days after transplantation the tumor mass was excised and weighed. At the same time, blood was sampled to perform hematologic and biochemical tests for safety assessment.

Statistical analysis

Between-group comparisons were made using the *t*-test, with a significance level of $p < 0.05$.

Results

Pharmacodynamic study

Serum 5-FU concentration–time profiles in the 5-FU monotherapy group and the 5-FU plus cisplatin combination group are shown in Figure 1. No significant differences were observed between the two groups at any time ($p = 0.06–0.85$), although the value of 0.5 h was lower in the 5-FU monotherapy group than in the combination group [1.5 (0–3) versus 29 (22–36) ng/ml]. Steady-state blood 5-FU concentrations were reached by 12 h after the start of continuous infusion.

The time-course of the 5-FU level in tumor tissue in the two groups is shown in Figure 2. No significant between-group differences were observed ($p = 0.14–0.72$) at any time except at 1 h, when the tissue level in the 5-FU monotherapy group was

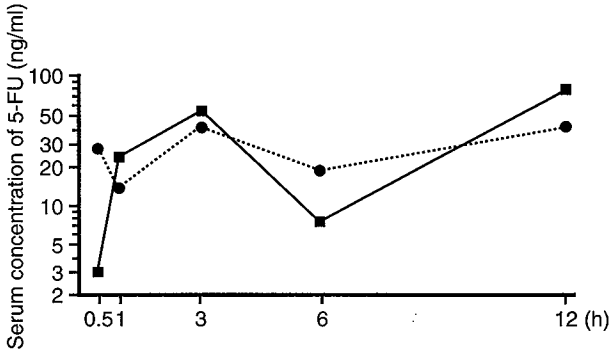

Figure 1. Serum concentration of 5-FU over time. ■: 5-FU monotherapy. Each point represents the mean of duplicate assays performed on the combined samples of three mice. ●: 5-FU + low-dose cisplatin. Each point represents the mean of duplicate assays performed on the combined samples of three mice.

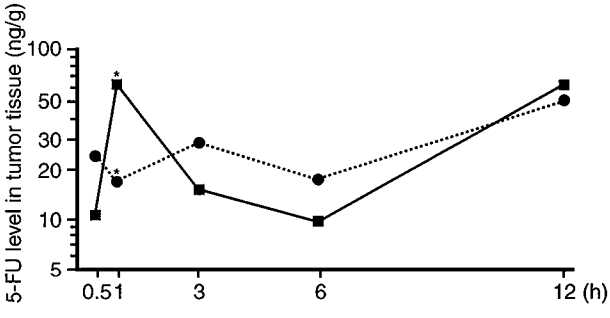

Figure 2. 5-FU level in tumor tissue over time. ■: 5-FU monotherapy. Each point represents the mean of duplicate assays performed on the combined samples of three mice. ●: 5-FU + low-dose cisplatin. Each point represents the mean of duplicate assays performed on the combined samples of three mice. * $p < 0.05$ (*t*-test).

significantly higher than that in the combination group [62.5 (62–63) versus 17.0 (12–22) ng/g]. In both groups, relatively high drug levels were obtained at 12 h (47.5 and 60.5 ng/g, respectively).

Table 1 compares the $AUC_{0-12\,h}$ for 5-FU in the serum and tumor tissue between the two groups. The serum 5-FU $AUC_{0-12\,h}$ in the combination group was lower than that in the monotherapy group, but the difference was not statistically significant ($p = 0.6804$). The 5-FU $AUC_{0-12\,h}$ in tumor tissue did not differ significantly between the two groups, indicating that concomitant cisplatin had practically no effect on the penetration of 5-FU into tumor tissue.

Antitumor effect

Treated:control ratios of tumor weights (T:C) determined 8 days after transplantation are shown in Figure 3. 5-FU monotherapy exerted a slight antitumor effect, while low-dose cisplatin monotherapy had no noticeable antitumor effect. In contrast, 5-FU plus cisplatin combination therapy achieved a favorable tumor response and the T:C value was significantly lower than those in the untreated control and cisplatin monotherapy groups ($p < 0.05$), although it was comparable to that obtained in the 5-FU monotherapy group ($p = 0.2529$).

Table 2 shows the laboratory values obtained in each treatment group. No significant between-group differences were observed in hematologic parameters [platelet, red blood cell (RBC) or white blood cell (WBC) counts] or in blood biochemical variables (blood urea nitrogen, serum glutamic pyruvic transaminase or serum glutamic oxalacetic transaminase levels). However, the mean WBC count in the 5-FU plus cisplatin group was slightly and not significantly lower than those obtained in the other three groups ($p = 0.22–0.49$).

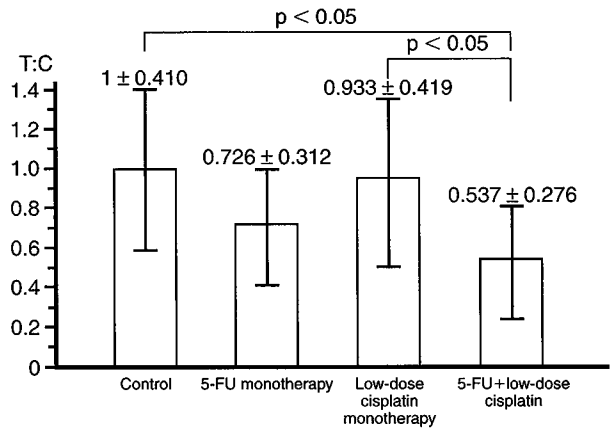

Figure 3. Antitumor effect (T:C ratio). Each point represents the mean ± SD of seven mice.

Discussion

This preliminary study of 5-FU infusion plus low-dose cisplatin was performed using an animal model of continuous 5-FU infusion. The Alzet micropump used for continuous i.p. 5-FU infusion is a capsule-type osmotic pump that is designed to deliver a drug solution at a constant rate when placed in the body. The doses of 5-FU and cisplatin used in the study of tumor response are equivalent to the respective clinical doses used in our department. The two agents have been reported to interact with each other *in vitro* when combined in a ratio of 1:5 (v/v) to reduce the residual potency of cisplatin;[12] however, combination of the two agents does not alter the potency of 5-FU. Based on previous findings, combination of a bolus injection of aqueous cisplatin solution with continuous infusion of 5-FU is unlikely to alter the potency of 5-FU and may have only a negligible effect on the potency of cisplatin because of the scarcity of 5-FU relative to cisplatin.

Thus, this experimental model simulated well the

Table 1. $AUC_{0-12\,h}$ [(ng/ml) h] of 5-FU during continuous infusion in mice administered 5-FU alone or in combination with cisplatin [mean of duplicate assays performed on combined tumor and serum samples from three mice (range)]

Sample	5-FU monotherapy	5-FU + low dose cisplatin	*t*-test
Serum	435.88 (259.50–612.25)	342.75 (259.00–426.50)	NS ($p = 0.6804$)
Tumor tissue	345.13 (286.75–403.50)	323.75 (258.00–389.50)	NS ($p = 0.8306$)

$AUC_{0-12\,h}$ = area under the serum or tumor tissue drug concentration–time curve 0–12 h after the start of infusion; NS = not significant.

Table 2. Hematologic values in mice 8 days after i.p. administration of continuous 5-FU 10 mg/kg/day, cisplatin 0.2 mg/kg/day or continuous 5-FU 10 mg/kg/day plus cisplatin 0.2 mg/kg/day and in untreated mice

Parameter	Control	5-FU monotherapy	Low-dose cisplatin monotherapy	5-FU + low-dose cisplatin
Platelets[a] ($\times 10^3/mm^3$)	37.10 ± 6.44	41.48 ± 17.93	36.43 ± 5.80	33.33 ± 9.42
Red blood cells[a] ($\times 10^3/mm^3$)	869.75 ± 27.49	771.25 ± 189.95	928.00 ± 66.01	811.33 ± 243.48
White blood cells[a] (no. cells/μl)	8275 ± 3036	9500 ± 4416	9525 ± 2689	6533 ± 2948
Blood urea nitrogen[b] (mg/dl)	25.78 ± 2.13	21.40 ± 5.44	27.40 ± 4.36	22.80 ± 3.01
SGOT[b] (IU/l/37°C)	300.8 ± 62.90	329.8 ± 39.80	336.6 ± 48.20	363.0 ± 74.90
SGPT[b] (IU/l/37°C)	44.0 ± 8.60	40.0 ± 1.41	45.6 ± 4.56	45.8 ± 9.44

[a]Mean ± SD in four mice.
[b]Mean ± SD in four mice.
SGOT = serum glutamic oxalacetic transaminase; SGPT = serum glutamic pyruvic transaminase.

clinical setting of the continuous 5-FU infusion plus low-dose cisplatin regimen, and accurately reflected the clinical efficacy and safety of the combination chemotherapy. The high susceptibility of Sarcoma 180 to 5-FU and cisplatin was demonstrated *in vivo* by Ishiyama *et al.*[13] and Rosenberg *et al.*,[14] who reported a 57.1% suppression of tumor growth by i.p. 5-FU 500 μg/day for 10 consecutive days and a T:C ratio of 0.32 achieved with daily i.p. cisplatin 1.25 mg/kg/day (equivalent to its standard clinical dose) given for 10 consecutive days. The dose of cisplatin used in the present study seemed to be insufficient for achieving a significant tumor response. This indicates that concomitant cisplatin given at a dose that was inefffective by itself tended to enhance the antitumor effect of 5-FU.

The synergism of the antitumor activities of 5-FU and cisplatin was first suggested from the biochemical aspect by Scanlon *et al.* in their *in vitro* study[1] and was subsequently established *in vivo* by Shirasaka *et al.*[2,3] 5-FU exerts an antitumor effect by mediation of the following two metabolites: 5-fluorouridine triphosphate (FUTP), which is incorporated into RNA and causes RNA dysfunction, and 5-fluorodeoxyuridine monophosphate (FdUMP), which forms a covalent ternary complex with thymidylate synthase (TS) and 5,10-methylene-tetrahydrofolate (5,10-CH_2-THF), leading to the inhibition of methylation of deoxyuridine monophosphate (dUMP). The antitumor effect of cisplatin is based on direct and indirect inhibition of DNA synthesis, i.e. via the binding of free platinum to two guanine molecules in DNA and the inhibition of intracellular methionine uptake at the cell membrane by protein-binding platinum. Scanlon *et al.* observed that cisplatin inhibited methionine uptake by tumor cells *in vitro*, which induced intracellular methionine synthase in association with activation of folate metabolism, leading to increased intracellular formation of tetrahydrofolate (THF) and 5,10-CH_2-THF.[1] They postulated that by this mechanism cisplatin may facilitate a greater supply of folate derivatives for FdUMP (an active metabolite of 5-FU) and thus form covalent ternary complexes with TS, thereby enhancing the antitumor effect of 5-FU. Shirasaka *et al.* also reported that *in vivo* cisplatin increased the pool of reduced folates (THF and 5,10-CH_2-THF) about 2- to 3-fold in tumor cells and that application of 5-FU to cisplatin-treated tumor cells significantly reduced [^{3}H]deoxyuridine incorporation into DNA,[2,3] a finding suggestive of a strong inhibition of TS by FdUMP. Based on these findings, the authors suggested that cisplatin might enhance the FdUMP-mediated antitumor effect of 5-FU by blocking the methionine supply to tumor cells and inducing intracellular methionine synthesis, which leads to the activation of folate metabolic processes [conversion of 5-methyltetrahydrofolate (CH_3-THF) to THF and to 5,10-CH_2-TNF]. In the present pharmacodynamic study, concomitant use of low-dose cisplatin had no significant effect on the time-course of the 5-FU level in the serum or tumor tissue, particularly on the $AUC_{0-12\,h}$ in tumor tissue. Therefore, the postulated synergism between 5-FU and cisplatin cannot be explained by any pharmacodynamic interaction between the two agents and might be attributable solely to the above-mentioned biochemical interaction.

Cisplatin generally causes severe renal toxicity, which can be prevented by adequate hydration before and after use of the drug. Low-dose cisplatin therapy has caused only mild toxicity, with no associated renal toxicity, and it has been well tolerated, with no need for hydration.[8–11] Therefore, continuous 5-FU infusion plus low-dose daily cisplatin may become a practical regimen if its efficacy is established. In the present study, concomitant administration of cisplatin at 0.2 mg/kg/day tended to

enhance the antitumor effect of 5-FU given at 10 mg/kg/day by continuous infusion without affecting laboratory parameters. Thus, the results provide preliminary experimental evidence for the clinical efficacy and application of continuous 5-FU infusion plus low-dose daily cisplatin. The proposed mechanism of the synergism between the two agents (i.e. biochemical modulation) should be studied in more detail using this model. Studies of the dose–response relationship, starting with the doses used in the present study, and on the optimal dose ratio of the two agents are now ongoing.

Conclusion

Continuous 5-FU infusion plus low-dose daily cisplatin has now been clinically tried because of its moderate anti-tumor efficacy in gastric and colorectal cancers and its low toxicity. In the present study, we investigated the efficacy of the 5-FU/cisplatin combination using tumor-bearing mice transplanted with Sarcoma 180. When 5 day continuous infusion of 5-FU (10 mg/kg/day via an i.p. implanted micropump) was combined with cisplatin (1 mg/kg, given as a bolus i.p. injection), there were no significant differences in the time-course changes of the serum and tumor tissue levels of 5-FU, as compared with those obtained with 5-FU monotherapy. On the other hand, when the same 5-FU regimen was combined with 5 day continuous i.p. infusion of cisplatin (0.2 mg/kg/day), the anti-tumor efficacy and leukocyte reduction tended to be enhanced as compared with 5-FU monotherapy. The findings of the present study indicate that the combination of low-dose cisplatin may modulate the pharmocodynamics of 5-FU.

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