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Metabolic basis of the synergistic antitumor activities of 5-fluorouracil and cisplatin in rodent tumor models in vivo

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Abstract. The biochemical mechanism of the synergy of 5-fluorouracil (FUra) and cisplatin (CDDP) was studied using transplantable tumors in rodents in vivo. The reduced folate 5,10-methylenetetrahydrofolate (CH_2FH_4) and its precursor tetrahydrofolate (FH_4) are essential cofactors for the formation of a tight ternary complex of thymidylate synthase (TS) and 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) derived from FUra. Intraperitoneal administration of CDDP (5 mg/kg) inhibited the incorporation of exogenous L-methionine into ascitic tumor cells and increased the levels of CH_2FH_4 and FH_4 in ascitic Yoshida sarcoma and P-388 cells transplanted into rats and mice to levels about 2–3 times those measured in cells from animals that were not treated with CDDP. Preincubation with 10^{-6} *M* FUra in Hanks' medium inhibited [6-^{3}H]-2′-deoxyuridine incorporation into DNA of tumor cells from CDDP-treated rats 3 times more than that into cells from untreated rats, indicating that the inhibition of TS by FdUMP derived from FUra was enhanced in the presence of CH_2FH_4. Intraperitoneal administration of CDDP on day 1 and continuous infusion of FUra from day 1 to day 6 had synergistic effects in inhibiting tumor growth in Yoshida sarcoma-bearing rats. Oral administration of UFT, a combined form of 1 *M* tegafur and 4 *M* uracil, for 7 consecutive days beginning at 24 h after tumor implantation and a single i.p. injection of CDDP on day 1 had a significantly greater effect than did either agent alone. These results suggest that CDDP significantly enhances FUra cytotoxicity by inhibiting intracellular L-methionine metabolism and consequently increasing the reduced folate pool in mammalian tumor models in vivo.

Introduction

Extensive clinical trials have been conducted on administration schedules of anticancer drugs alone and in combination with other antitumor agents or noncytotoxic modulators in attempts to increase the clinical response rates of cancer patients. 5-Fluorouracil (FUra), synthesized and characterized by Duschinsky et al. [6], has been widely used for treatment of gastrointestinal, gastric, breast, head and neck, and genitourinary cancers. On the basis of recent findings, its administrations by continuous venous infusion (CVI) at low doses over a long period and at high doses for 5 days are now standard procedures for treatment of patients with advanced and metastatic colorectal carcinoma and squamous-cell carcinoma of the head and neck, respectively [15, 19].

Various other drugs and noncytotoxic modifiers have been applied in combination with FUra to obtain a higher response rate and longer survival of patients. To date, the most effective regimens have been the combinations of CVI FUra and cisplatin (CDDP) or leucovorin. In particular, 5-day CVI FUra plus CDDP has resulted in high response rates (60%–90%) in patients with metastatic head and neck cancers [11, 16, 27, 38, 39], and long-term CVI FUra plus CDDP has resulted in objective responses (40%–60%) of metastatic colorectal cancers [2, 5, 20], whereas FUra and CDDP given singly are reported to be less effective.

The reasons for the cytotoxic effect of FUra are widely accepted to be, first, that 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), an active metabolite of FUra, inhibits thymidylate synthase (TS) [12, 18, 28, 33, 34], and second, that FUra is incorporated into RNA and distorts gene expression [4, 22, 41]. On the other hand, the effect of CDDP is thought to be due mainly to its inhibition of DNA synthesis by tight binding to guanine residues of double-stranded DNA [1].

The high response rate of cancer patients to CVI FUra plus CDDP may be due to a reciprocal relationship of the cytotoxic actions of FUra and CDDP. In this connection, it is noteworthy that Scanlon et al. [29, 30] reported that CDDP inhibited the transport of neutral amino acids, including L-methionine, into murine L1210 cells and also induced an elevation of the intracellular levels of reduced folates such as 5,10-methylenetetrahydrofolate (CH_2FH_4)

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and tetrahydrofolate (FH_4), whereby the increased level of CH_2FH_4 resulted in a 2.5-fold increase in the binding of FdUMP to TS. However, it is uncertain whether an increase in reduced-folate levels in tumor cells was observed in the tumor-bearing animals treated with CDDP or contributed to the antitumor activity of FUra plus CDDP.

The present paper concerns studies on the changes in reduced-folate levels in ascitic tumor-bearing rodents in vivo following administration of CDDP and on the contribution of elevated levels of reduced folates to the antitumor activity of FUra or its derivative.

Materials and methods

Drugs. FUra was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). CDDP was obtained from Bristol-Myers Co. (Wallingford, Conn., USA). UFT [7, 14], which is a combined form of 1 *M* tegafur and 4 *M* uracil and is given orally for the treatment of cancer patients, was a product from Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan). [6-^{3}H]-2′-Deoxyuridine (dUrd; 1,110 GB9/mmol), [methyl-^{14}C]-L-methionine (L-Met; 2.1 GBq/mmol), and [6-^{3}H]-5-fluoro-2′-deoxyuridine (FdUrd; 662 GBq/mmol) were purchased from DuPont Co. (USA). 5-Chloro-2,4-dihydroxypyridine (Cl-DHP) was synthesized in our laboratories. All other chemicals used were commercial products.

Tumor cells. Yoshida sarcomas and P-388 leukemia cells, maintained in our institute, were passaged in male Donryu-strain rats and male DBA/2 mice, respectively, by i.p. inoculation at weekly intervals.

Preparation of [6-^{3}H]-FdUMP. [6-^{3}H]-FdUMP was enzymatically converted from [6-^{3}H]-FdUrd by mammalian thymidine kinase. Thymidine kinase was extracted and partially purified from Yoshida sarcoma cells. In all, 2 g of the frozen cells were homogenized with 4 vol. 50 m*M* TRIS-HCl (pH 8.0) containing 250 m*M* sucrose, 5 m*M* $MgCl_2$, and 1 m*M* dithiothreitol, and the homogenate was centrifuged at 105,000 *g* for 60 min. Thereafter, 3.12 g solid ammonium sulfate was gently added to 8 ml supernatant fluid. The mixture was stirred for 2 h at 4° C and then centrifuged at 9,000 *g* for 20 min. The precipitate was dissolved in 2 ml homogenizing buffer and dialyzed against the same buffer overnight. After centrifugation of the dialyzate, the resultant supernatant was used as partially purified thymidine kinase.

Next, 37.0 MBq [6-^{3}H]-FdUrd (662 GBq/mmol) in an aqueous solution was evaporated under vacuum at 40° C and then dissolved in 0.4 ml 25 m*M* TRIS-HCl (pH 8.0) containing 25 m*M* adenosine 5′-triphosphate (ATP) and 30 m*M* α-glycerolphosphate. Then, 1.6 ml purified thymidine kinase solution was added to the [6-^{3}H]-FdUrd solution, and the mixture was incubated at 37° C for about 2 h. Almost all of the [6-^{3}H]-FdUrd was converted to [6-^{3}H]-FdUMP within 2 h. The reaction was stopped by the addition of 1 ml 20% trichloroacetic acid (TCA) and 2 ml distilled water at 0° C. Following centrifugation (3,000 rpm), the supernatant was shaken for 10 min with an equivolume of 17.7% (v/v) tri-*n*-octylamine in 1,1,2-trichloro-1,2,2-trifluoroethane and then centrifuged. After evaporation of the aqueous layer, the residue was dissolved in few volumes of water and applied to a polyethyleneimino-cellulose plate (5 × 10 cm). The plate was developed with a mixture of 1 *M* acetic acid and 1 *M* lithium chloride. Thereafter, a portion of the [6-^{3}H]-FdUMP was sacrificed and it was eluted from the resin with 0.2 *M* ammonium formate. The eluate was diluted to a concentration of 500 pmol/ml with water.

Preparation of TS from Yoshida sarcoma cells. TS was extracted and partially purified from Yoshida sarcoma cells. In all, 5 g frozen cell pellets was homogenized with 3 vol. 200 m*M* TRIS-HCl (pH 7.4) containing 20 m*M* β-meraptoethanol, 15 m*M* cytidine 5′-monophosphate, and 100 m*M* sodium fluoride and then centrifuged at 10,500 *g* for 60 min. The supernatant was treated with ammonium sulfate (0–50% saturation) and centrifuged at 9,000 *g* for 20 min. Thereafter, the resultant precipitate was dissolved in 5 ml of the same buffer and dialyzed against the same buffer for 24 h. Partially purified TS was found to be capable of binding 25.2 ± 1.1 pmol [6-^{3}H]-FdUMP/ml.

Measurement of reduced folates. Levels of the intracellular reduced folates 5,10-methylenetetrahydrofolate (CH_2FH_4) and tetrahydrofolate (FH_4) were determined by a modification of the method of Priest et al. [25]. Tumor cells (about 2×10^7 cells/ml) were treated with 1 ml ice-cold 10% TCA and centrifuged at 3,000 rpm for 5 min. The resultant supernate was promptly shaken with 1 ml 17.7% (v/v) tri-*n*-octylamine in 1,1,2-trichloro-1,2,2-trifluoroethane for 5 min and the mixture was centrifuged. An aliquot (0.05 ml) of the aqueous layer was added to a reaction mixture consisting of 0.05 ml 2% bovine serum albumin, 0.05 ml [6-^{3}H]-FdUMP (500 pmol/ml) with or without 0.05 ml 26 m*M* formaldehyde, and 0.05 ml of the TS solution from Yoshida sarcoma cells, and the mixture was incubated at 30° C for 20 min. Then 10% TCA was added and acid-precipitable materials, obtained by centrifugation, were washed twice with 5% TCA and solubilized in concentrated formic acid. The radioactivity of CH_2FH_4-FdUMP-TS complexes was measured in a liquid scintillation counter. When formaldehyde is absent in the assay system, only the CH_2FH_4 contained in the preparation binds to the TS-FdUMP complex, whereas is the presence of formaldehyde, the FH_4 in the sample is easily converted to CH_2FH_4. Therefore, the FH_4 content of the preparation can be calculated from these two results.

Uptake of L-Met into ascitic tumor cells. Ascitic Yoshida sarcoma cells were collected from the intraperitoneal cavity of rats, washed, and resuspended in saline. In all, 1×10^7 cells of Yoshida sarcoma were incubated with 1 μ*M* [methyl-^{14}C]-L-Met (18.5 kBq) at 37° C for 10 min. Then, the cells were immediately washed with 4 ml ice-cold saline and centrifuged at 900 g for 5 min. This procedure was repeated once more. Next, 1 ml ice-cold 5% TCA was added to the cell pellets, and the mixture was stirred and centrifuged at 3,000 rpm for 10 min. The radioactivity of the supernatant was measured as described above.

2′-Deoxyuridine incorporation into DNA. Tumor cells obtained from the peritoneal cavity of rats were washed with saline, suspended in Hanks' medium that did not contain exogenous folic acid, and preincubated with 10^{-6} to 10^{-8} *M* FUra. They were then incubated with [6-^{3}H]-dUrd (5×10^{-7} *M*, 185 kBq) at 37° C. The DNA fraction of the cells was then extracted by the method of Schneider [31] and the radioactivity of the [6-^{3}H]-dUrd incorporated into the DNA was measured.

Antitumor activity. Solid-type Yoshida sarcomas were prepared by implanting 2×10^5 tumor cells into subepidermal tissues of the backs of rats on day 0. CDDP was injected i.p. on day 1, and FUra dissolved in saline was infused continuously for 6 consecutive days starting at 24 h after tumor implantation. Control rats received continuous infusions of saline alone. On day 7, the rats were killed and the tumors were removed and weighed. The antitumor activity (IR, in percent) was evaluated by the following formula:

$$IR(\%) = 1 - \frac{\text{mean tumor weight in drug-treated rats}}{\text{mean tumor weight in control rats}} \times 100$$

In the case of oral administration of UFT, 10–20 mg/kg UFT suspended in 5% acacia solution was given daily for 7 consecutive days starting at 24 h after the implantation of tumor cells. CDDP was injected i.p. on day 1. Control rats were given 5% acacia solution alone on the same schedule. On day 8, the antitumor activity was evaluated by the method described above.

Results

Uptake of [methyl-^{14}C]-L-Met by ascitic tumor cells from Yoshida sarcoma-bearing rats after CDDP administration

For examination of the effects of CDDP on the L-Met and folate pools of tumor cells. Yoshida sarcoma was transplanted i.p. into rats and CDDP was injected i.p. at 1, 2,

Table 1. Uptake of L-Met by ascitic tumor cells from Yoshida sarcoma-bearing rats after i.p. administration of CDDP

Dose of CDDP (mg/kg)	Rats (n)	L-Met uptake (pmol 1×10^{-7} cells min^{-1})	% Inhibition
Saline	4[a]	18.4 ± 2.9[b]	–
1.0	4	16.3 ± 0.3	11.4
2.0	4	15.8 ± 4.5	14.4
5.0	4	10.8 ± 1.9	41.3

[a] Rats were treated i.p. with CDDP on day 8 aftert the inoculation of Yoshida sarcoma cells

[b] Tumor cells were incubated with 1 μM L-Met (18.5 KBq) for 10 min in the assay mixture. Data represent mean values ± SD

Table 2. Effect of i.p. CDDP administration on intracellular levels of reduced folates in P388-bearing mice and Yoshida sarcoma-bearing rats

Tumor cells	CDDP (mg/kg)	Reduced-folate pool (pmol/2×10^7 cells)[a]	
		CH_2FH_4	FH_4
P388	–	0.075 ± 0.004	0.111 ± 0.004
	2.5	0.108 ± 0.013	0.172 ± 0.036
	5.0	0.152 ± 0.017	0.426 ± 0.200
Yoshida sarcoma	–	0.065 ± 0.004	0.190 ± 0.004
	5.0	0.139 ± 0.007	0.301 ± 0.055

Tumor cells were isolated from mice or rats at 24 h after the administration of CDDP. CH_2FH_4, 5,10-Methylenetetrahydrofolate; FH_4, tetrahydrofolate

[a] Data represent mean values ± SD

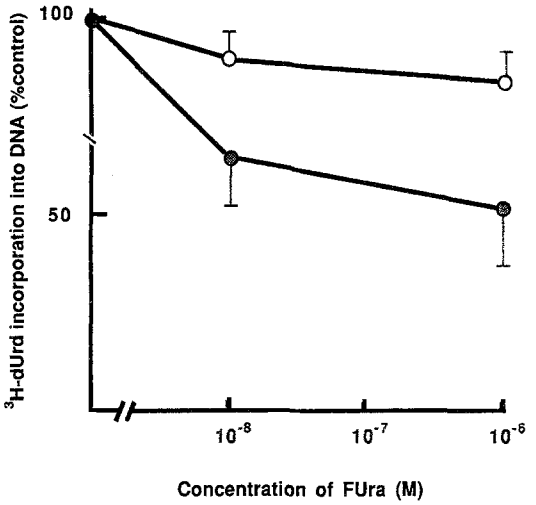

Fig. 1. Effect of preincubation with FUra on [6-^{3}H]-dUrd incorporation into DNA of Yoshida sarcoma cells in CDDP-treated and untreated rats. Tumor cells were obtained from untreated ascitic Yoshida sarcoma-bearing rats (○) and from those treated i.p. 24 h previously with 5 mg/kg CDDP (●). The cells were suspended in Hanks' solution that did not contain folic acid and were preincubated with 10^{-6} to 10^{-8} *M* FUra at 37° C for 10 min. They were then incubated with [6-^{3}H]-dUrd (5×10^{-7}, 185 kBq) for 10 min and the incorporation of [6-^{3}H]-dUrd into DNA was measured. Data represent mean values ± SD for 3 rats

and 5 mg/kg into the tumor-bearing rats on day 8. After 6 h, the cells were removed and suspended in Hanks' medium that did not contain exogenous folic acid. The cells were then treated with [methyl-^{14}C]-L-Met (1 μ*M*, 18.5 kBq) for 10 min and the intracellular L-Met level was determined. As shown in Table 1, the L-Met level in cells treated with 5 mg/kg CDDP was significantly decreased, suggesting that CDDP inhibited L-Met uptake by the cells.

Levels of reduced folates in ascitic tumors of CDDP-treated rodents

On day 8 after the implantation of ascitic tumor cells, rats and mice were treated i.p. with 2.5–5 mg/kg CDDP and 24 h later the tumor cells were collected and their contents of the reduced folates CH_2FH_4 and FH_4 were measured. As shown in Table 2, the CH_2FH_4 and FH_4 levels in ascitic P-388 cells from mice treated with 5 mg/kg CDDP were about 2 and 4 times, respectively, those of cells from mice that were not treated with CDDP. Treatment with CDDP at a dose of 2.5 mg/kg resulted in no significant increase in the CH_2FH_4 or FH_4 pool in the ascitic cells.

Similar results were obtained in ascitic Yoshida sarcoma-bearing rats treated with 5 mg/kg CDDP. The levels of CH_2FH_4 and FH_4 in CDDP-treated Yoshida sarcoma cells were about twice those in untreated cells. After 48 h, however, the reduced-folate levels in tumor cells from CDDP-treated rats were not significantly different from those in tumor cells from untreated rats, indicating that the elevated levels of reduced folates observed after 24 h decreased to normal levels within 48 h (data not shown).

Effect of FUra on incorporation of 2'-deoxyuridine into DNA in ascitic tumors of CDDP-treated rats

About 2×10^7 Yoshida sarcoma cells isolated from the intraperitoneal cavity of rats treated with CDDP at 5 mg/kg were suspended in Hanks' medium, preincubated with 10^{-8} and 10^{-6} *M* FUra in vitro, and then incubated with [6-^{3}H]-dUrd. As shown in Fig. 1, preincubation with 10^{-6} *M* FUra in vitro inhibited the incorporation of [6-^{3}H]-dUrd into the DNA fraction of CDDP-treated ascitic tumor cells by about 50% but inhibited that into untreated ascitic tumor cells by only about 15%. This finding suggests that the capacity for formation of the TS-FdUMP complex is enhanced by the increase in CH_2FH_4 and the consequent increase in the inhibition of DNA synthesis by the cells. These results indicate an increase in the antitumor activity of 5-FU.

Antitumor activity of a combination of i.p. CDDP and continuous intravenous infusion of FUra

In combination therapy, the most important factor is the optimal schedule for administration of the effector FUra. Recently, we observed that the plasma level of FUra remained nearly constant during continuous venous infusion (CVI) of FUra plus an inhibitor of FUra degradation [8] and thus could determine the relationship between levels of FUra in the blood of rats and its antitumor effect on Yoshida sarcoma.

Table 3. Antitumor effect of the combination of CDDP and continuous venous infusion of FUra on Yoshida sarcomas in rats

FUra[a] (mg/kg daily)	CDDP[b] (mg/kg)	Rats (*n*)	Tumor wt.[c] (g)	IR[d] (%)	Body wt. (g)[c]	
					Start	End
–	–	9	1.58 ± 0.43	–	236 ± 9	258 ± 11
–	5.0	8	1.11 ± 0.23	29.7	235 ± 9	234 ± 17
1.0	–	9	1.82 ± 0.81	0	239 ± 7	262 ± 11
1.0	5.0	8	0.40 ± 0.23	74.7	238 ± 7	212 ± 20
2.0	–	8	0.94 ± 0.18	40.5	239 ± 7	245 ± 13
2.0	5.0	8	0.07 ± 0.15	95.6	238 ± 7	204 ± 30

[a] FUra combined with a 10-fold molar ratio of Cl-DHP (11.2 and 22.4 mg/kg, respectively) was continuously infused from day 1 to day 7
[b] CDDP was injected i.p. on day 1 of the therapeutic periods (7 days)
[c] Data represent mean values ± SD
[d] Inhibition rate of tumor growth

Table 4. Antitumor effect of a combination of CDDP and a FUra derivative, UFT, on Yoshida sarcoma in rats

UFT[a] (mg/kg daily)	CDDP[b] (mg/kg)	Rats (*n*)	Tumor wt.[c] (g)	IR[d] (%)	Body wt. (g)[c]	
					Start	End
–	–	16	3.18 ± 0.87	–	237 ± 14	296 ± 16
–	5	6	2.48 ± 0.51	22.0	249 ± 7	293 ± 19
10	–	8	2.62 ± 0.63	17.6	241 ± 7	298 ± 11
10	5	8	2.20 ± 0.34	30.8	253 ± 5	283 ± 16
20	–	8	2.00 ± 0.52	37.1	242 ± 12	286 ± 17
20	5	7	0.57 ± 0.33	82.1	249 ± 14	238 ± 21

[a] UFT, a combined form of 1 *M* tegafur and 4 *M* uracil, was given to rats orally for 7 consecutive days. Values in the table represent doses as tegafur
[b] CDDP was injected i.p. on day 1 of the therapeutic periods (8 days)
[c] Data represent mean values ± SD
[d] Inhibition rate of tumor growth

We examined whether the increase in the intracellular level of reduced folates induced by CDDP administration contributes to the antitumor activity of FUra in combined treatment with a single i.p. injection of CDDP (5 mg/kg) and continuous infusion of FUra (1–2 mg/kg) for 6 days in rats with solid-type Yoshida sarcomas. FUra combined with Cl-DHP, a potent inhibitor of dihydrouracil dehydrogenase [EC 1.3.1.2], was used to maintain a constant blood level of 5-FU by inhibiting rapid FUra degradation in the liver [37]. Cl-DHP did not interact with CDDP in vitro or in vivo. As shown in Table 3, tumor growth was inhibited only 30% by CDDP alone and only about 10% and 35% by continuous infusion of FUra alone at 1 and 2 mg/kg, respectively. CDDP plus FUra had more antitumor activity than did either drug alone; 5 mg/kg CDDP plus infusion of FUra at 2 mg/kg daily inhibited tumor growth by about 96%. However, side effects as judged by the inhibition of increases in body weight also tended to increase with increasing antitumor activity.

Antitumor activity of a combination of UFT and CDDP

The effect of UFT, which is now widely used clinically in Japan [7, 14, 21], in combination with CDDP was examined in Yoshida sarcoma-bearing rats. UFT (10 or 20 mg/kg) was given orally for 7 consecutive days beginning at 24 h after tumor implantation, and CDDP (5 mg/kg) was injected i.p. once on day 1. As shown in Table 4, the effect of UFT at 20 mg/kg plus CDDP at 5 mg/kg was significantly greater than that of either drug alone.

Discussion

Two approaches have been used in studies on how to potentiate the clinical antitumor effectiveness of FUra. One attempts to establish the optimal dose and time schedule. Results have shown that long-term continuous infusion of a low dose of FUra is more effective in attaining a high response rate than is the conventional bolus schedule [3, 32, 36]. Lokich et al. [19] demonstrated that continuous infusion of FUra at a dose of 300 mg/m^2 gave a higher response rate than did the conventional bolus schedule in a prospective randomized study in patients with metastatic colorectal carcinoma. The other approach is biochemical modulation of FUra. Various modulators have been used to potentiate FUra cytotoxicity in preclinical and clinical trials. Two main explanations have been given for the cytotoxicity of FUra. One widely accepted explanation is that FdUMP, an active metabolite of FUra, inhibits TS (EC 2.1.1.45) covalently [12, 18, 28, 33, 34]. The other explanation is that FUra is incorporated into RNA and distorts gene expression [4, 22, 41]. Therefore, it is thought that modulation of these two metabolic effects of FUra is important for potentiating the cytotoxicity of FUra.

Recently, Spears et al. [35] studied the mechanism of innate resistance to FUra in 37 patients with colorectal carcinoma who were given bolus injections of FUra. They measured the degree of TS inhibition and the levels of FdUMP and dUMP and concluded that in about 35% of 30 patients, resistance to FUra was due to an insufficient intracellular reduced-folate pool. Thus, an increase in the intracellular CH_2FH_4 level may enhance the inhibition of TS by FdUMP derived from FUra.

In 1983, Scanlon et al. [29] reported that CDDP inhibited the incorporation of L-methionine into L1210 leukemia cells in vitro by inhibiting membrane-bound Na^+/K^+-ATPase. Moreover, these investigators [30] showed that the intracellular levels of CH_2FH_4 and FH_4 in human ovarian A2780 cells markedly increased after treatment with CDDP and that treatment with 5-fluoro-2′-deoxyuridine (FdUrd) increased the binding capacity of the TS-FdUMP complex in the cells. As the intracellular level of reduced folate changes according to extra- or intracellular methionine metabolism [17], CDDP seems not only to bind to guanine residues in DNA molecules but also to affect the biochemical modulation of FUra. However, there is no report as to the elevation of intratumoral reduced folates in tumor-bearing animals treated with CDDP and its contribution to the antitumor activity of FUra.

Therefore, we investigated the possible biochemical modulation of FUra by CDDP in rodent tumor models in

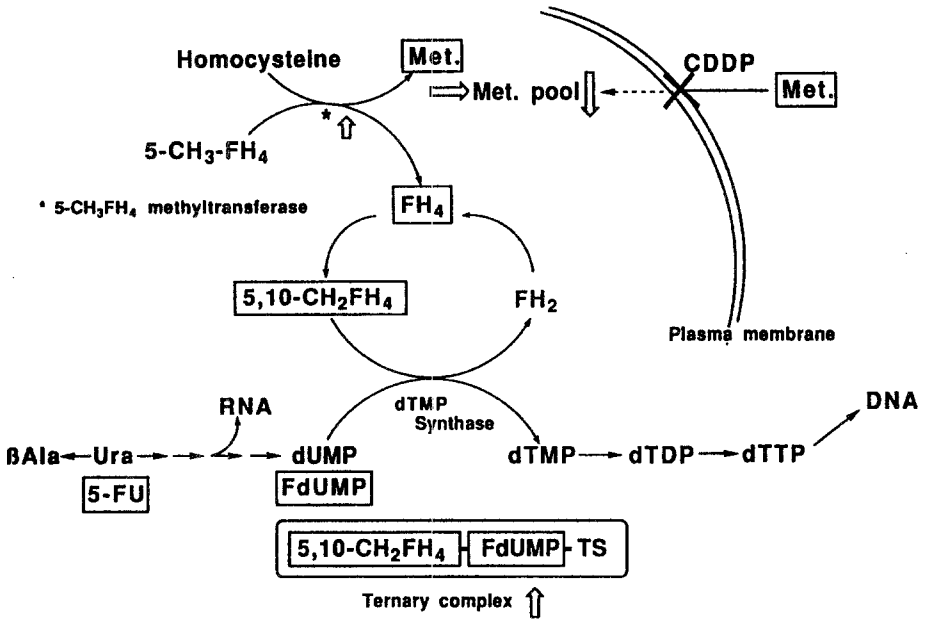

Fig. 2. Mechanism of the biochemical modulation of FUra by CDDP

vivo. We mainly used the maximal tolerable dose of CDDP (5 mg/kg) in our 8-day experiments (data not shown). After i. p. injection of CDDP into ascitic Yoshida sarcoma-bearing rats, the cellular uptake of exogenous L-Met by the tumor cells was significantly lower than that by cells from untreated rats, and the intracellular pools of reduced folates such as CH_2FH_4 and FH_4 were about twice those in cells from control rats. Similar elevations of the intracellular levels of reduced folates by CDDP were observed in P-388-bearing mice. These results suggest that the incorporation of dUrd into the DNA of tumor cells from CDDP-treated rats would be inhibited more strongly after 5-FU treatment. In treatment with FUra and consequent [6-^{3}H]-dUrd, we used Hanks' medium lacking exogenous folic acid to avoid an influence of exogenous folic acid on the intracellular folates in Yoshida sarcoma cells removed from the intraperitoneal cavity of rats treated with CDDP. After pretreatment with FUra (10^{-6} *M*), the incorporation of [6-^{3}H]-dUrd into DNA was inhibited about 50% in Yoshida sarcoma cells from CDDP-treated rats but only about 15% in tumor cells from untreated rats. These data show that pretreatment of rats with CDDP potentiated the inhibition of TS by FdUMP derived from FUra in the tumor. Moreover, in therapeutic experiments on rats bearing Yoshida sarcomas, CDDP treatment and continuous infusion of FUra and/or consecutive oral administration of UFT, a combined form of 1 *M* tegafur and 4 *M* uracil [7, 14], had synergistic antitumor effects.

There are reports of potentiation of the antitumor effect of FUra by CDDP in the treatment of colon tumors in CF_1 mice [23] and of human colorectal carcinoma xenografted into nude mice [24]. In these studies, the antitumor activity of FUra and CDDP in combination was found to be significantly higher for the sequence FUra→CDDP than for the opposite sequence.

In clinical studies on the treatment of squamous-cell carcinomas of the head and neck [11, 16, 27, 38, 39], colorectal carcinomas [2, 5, 20], and non-small-cell lung cancers [13, 26, 40], CDDP has generally been given by i.v. bolus injection or infusion at an early stage or at simultaneous periods in a week or one cycle during continuous infusion of 5-FU. Gonzalez-Brown et al. [9, 10] recently reported the results of a phase II clinical trial showing that oral tegaful was effective in combination with CDDP or carboplatin (CBDCA) in patients with head and neck carcinomas. For a combination of tegaful (1,000 mg/m^2 given for 21 days) and CDDP (100 mg given on day 1 only), the complete response (CR) rate was 22% and the partial response (PR) rate was 75%. Thus, the total response rate was 97%, which is very high.

Although various combination schedules have been reported as regards the potentiation of antitumor efficacy by the use of FUra plus CDDP, it is suggested that the elevation of response rates by FUra plus CDDP in cancer patients is based on the mechanism shown in Fig. 2. The present results support this functional mechanism of FUra and CDDP.

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