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Modulation of the Antitumour Activity of Cisplatin Alone and in Combination with 5-Fluoro-2′-deoxyuridine by *N*-phosphonacetyl-L-aspartate in Murine Colon Carcinoma No. 26

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Modulation of the therapeutic efficacy of cisplatin (CDDP) and 5-fluoro-2′-deoxyuridine (FdUrd) alone and in combination with *N*-phosphonacetyl-L-aspartate (PALA) was evaluated in mice bearing colon carcinoma (C-26) using a weekly intravenous (i.v.) push schedule for 3 weeks. A non-toxic dose of PALA (100 mg/kg) was administered i.v. 24 h prior to the i.v. administration of CDDP ± FdUrd. The maximum tolerated doses (MTD) of CDDP and FdUrd when used as a single agent were 9 and 400 mg/kg, respectively. In combination, however, the MTD of CDDP and FdUrd were 2.5 and 300 mg/kg, respectively. PALA did not significantly affect the MTD. PALA improved the antitumour activity of CDDP or FdUrd when used alone; however, the highest tumour response, 66% complete tumour regression, was achieved with a PALA modulation of CDDP and FdUrd in combination.

Key words: PALA, cisplatin, FdUrd, colon 26
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INTRODUCTION

N-PHOSPHONACETYL-L-ASPARTATE (PALA) is a specific transition state inhibitor of aspartate transcarbamylase (ACTase) essential for *de novo* pyrimidine nucleotide biosynthesis [1]. PALA can potentiate the antitumour activity of 5-fluorouracil (5-FU) and of 5-fluoro-2′-deoxyuridine (FdUrd) in animal systems and in patients [2–6]. PALA is ineffective when used as a single agent, but can reduce the cellular bioavailability of normal pyrimidine nucleotides by inhibition of ACTase and enhance the synthesis of the fluorinated pyrimidine nucleotides and incorporation into cellular RNA [2, 3, 7].

Cisplatin (CDDP), a DNA cross-linking agent, is also known to interact with 5-FU [8]. It is used widely as a single agent and in polychemotherapy for the treatment of patients with solid tumour malignancies. A clinical trial by Voigt and Kleeberg [9], using PALA, CDDP and vindesine in patients with advanced malignant melanoma demonstrated a high activity of this combination [24% complete regression (CR) and 19% partial response (PR)].

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Fluoropyrimidines in combination with CDDP are active in patients with cancer of the head and neck [10–12] and disseminated colorectal cancer [13]. The mechanism of the modulation of 5-FU by CDDP remains unclear, however. Modulation of repair of platinum–DNA adducts formation [14] and potentiation of the effect of 5-FU at the level of TS have been implicated [8, 15].

In this study, the ability of PALA to modulate the therapeutic efficacy of CDDP and FdUrd alone and in combination was evaluated in a murine colon carcinoma tumour with differential sensitivity to these agents.

MATERIALS AND METHODS

Drugs

PALA was obtained from U.S. Bioscience (West Conshohocken, Pennsylvania, U.S.A.), FdUrd from Sigma Chemical Co. (St Louis, Missouri, U.S.A.) and CDDP from Bristol Co. (Syracuse, New York, U.S.A.). All drugs were dissolved in 0.9% NaCl solution, pH 7.0, volume adjusted to 0.20 ml/20 g mouse weight on an mg/kg basis.

Mice, tumour transplant and antitumour activity

Six- to seven-week-old female BALB/c mice, obtained from Harlan (Prattville, Alabama, U.S.A.) were kept five per cage with water and food *ad libitum* according to an institution-approved animal protocol. Drugs were given by intravenous administration (i.v. push) as described previously [16]. PALA was given at a low and ineffective dose of 100 mg/kg, 24 h prior to therapy. Data reported were generated using a weekly ×3

schedule (wk ×3). Antitumour activity evaluation was carried out at the maximum tolerated doses (MTD) of the drug alone and in combination under conditions with and without modulation by PALA. Experiments were carried out at least in triplicate with at least five mice in each group.

Antitumour activities were evaluated using 6–9-week-old female BALB/c mice bearing subcutaneous (s.c.) colon no. 26 carcinoma [17]. Tumour volumes were determined three times a week, calculated as described previously [4, 18, 19]. Antitumour parameters included tumour doubling time (TD); tumour volume of treated over tumour volume of untreated groups (T/C) and PR representing a decrease in tumour volume > 50%; CR at 3 months are considered cures [4].

The MTD was defined as the highest dose that could be administered without causing drug-related deaths.

Statistical evaluation

Student's *t*-tests for unpaired data were used for statistical evaluation and differences were considered to be statistically significantly different when $P < 0.05$.

RESULTS

MTD

The MTDs of CDDP and FdUrd alone and in combination with PALA are outlined in Table 1. The data indicate that PALA did not potentiate the toxicity of FdUrd or CDDP alone or in combination. When combined, however, the doses of both agents had to be reduced. Since FdUrd is the active agent in colorectal cancer, the dose was reduced by 25% and the MTD of the combination was identified to be 2.5 mg/kg of CDDP with 300 mg/kg FdUrd. The effect of low-dose PALA (100 mg/kg/week ×3) on the antitumour activity of CDDP and FdUrd at the MTD (9 mg/kg/week ×3) and 300 mg/kg/week ×3, respectively, was investigated and the results are summarised in Table 1.

PALA significantly increased the CR and PR rates induced by FdUrd or CDDP alone or in combination. Modulation of the antitumour activity of FdUrd was greater with PALA than with CDDP. Although the combination of CDDP and FdUrd was more active than either agent alone, the highest antitumour activity was achieved when PALA was administered 24 h prior to the administration of CDDP and FdUrd in combination. The CR rate was increased from 25 to 66% (Table 1).

Table 1. *Antitumour activity of CDDP and FdUrd modulated by PALA: weekly ×3 schedule*

Treatment	Doses (mg/kg/week)	TD (days)	T/C_{max}	Response (% total) PR	CR
CDDP	9*	9.8 ± 1.9	0.2 ± 0.04	6	0
CDDP	2.5	4.7 ± 0.6	0.60 ± 0.08	0	0
PALA→CDDP	9*	25.9 ± 2.5	0.08 ± 0.01†	22	6
FdUrd	400	28.0 ± 3.2	0.08 ± 0.01	20	20
FdUrd	300	22.7 ± 3.4	0.16 ± 0.03	15	10
PALA→FdUrd	400*	36.3 ± 4.8	0.04 ± 0.02	30	50
FdUrd + CDDP	300* + 2.5	21.5 ± 3.8	0.09 ± 0.02	40	25
PALA→FdUrd +CDDP	300 + 2.5*	42.8 ± 2.2†	0.03 ± 0.01†	34	66

TD, tumour doubling time; T/C_{max}, maximum T/C; PR, partial response (> 50% reduction in tumour size); CR, complete tumour reduction (cures).

* MTD; maximum tolerated dose (no lethality). † Significantly different from CDDP and FdUrd alone.

DISCUSSION

The data in this paper demonstrate that the antitumour activity of FdUrd in mice bearing colon carcinoma no. 26 can be significantly improved by double modulation by CDDP in combination with PALA. PALA increased the therapeutic activity of CDDP and FdUrd alone, but the effect was most pronounced when CDDP and FdUrd were used in combination at their MTD doses.

The combination of fluoropyrimidines and CDDP is one of the most effective chemotherapeutic regimens in the treatment of patients with head and neck cancer. Response rates up to 72% have been reported [10–12]. The recent findings demonstrating that FdUrd is more effective than 5-FU in C-26 [4], and the unexpected high response rates in melanoma observed by Voigt and Kleeberg [9] provided the basis for evaluating the effects of PALA on the antitumour activity of CDDP and FdUrd. The observation that PALA can significantly enhance the antitumour activity of the CDDP/FdUrd combination by increasing CR rate (Table 1) is not totally surprising, as CDDP and FdUrd can both be modulated by PALA. The mechanism remains unclear, however, possible mechanisms include an increase in the extent of duration of TS inhibition [8], a consequence of reduced levels of the competing metabolite dUMP with FdUMP, or inhibition of DNA repair resulting from decreased pyrimidine nucleotide pools following treatment with PALA. These and other possibilities need to be evaluated in future investigations. If the effective modulation of CDDP and FdUrd by PALA in C-26 can be confirmed in other tumour models, this drug combination should be tested in patients with head and neck, colorectal and breast cancers. In fact, a phase I clinical trial with parallel laboratory investigation of PALA/CDDP is under evaluation at the RPCI [20]. Once the MTD and the toxicity have been defined, the possibility of a phase I clinical protocol of the combination of CDDP/FdUrd modulated by PALA will be considered in future clinical trials.

1. Jayaram H, Cooney DA, Vistica DT, Kariya S, Johnson RK. Mechanisms of sensitivity or resistance of murine tumor to *N*-(phosphonacetyl)-L-aspartate (PALA). *Cancer Treatment Rep* 1979, **63**, 1291–1302.
2. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Casper ES, Young CW. Therapeutic utility of utilizing low doses of *N*-(phosphonacetyl)-L-aspartic acid in combination with 5-fluorouracil: a murine study with clinical relevance. *Cancer Res* 1983, **43**, 2317–2321.
3. Spiegelman S, Sawyer RC, Nayak R, Ritzi E, Stolfi RL, Martin DS. Improving antitumor activity of 5-fluorouracil by increasing its incorporation into RNA via metabolic modulation. *Proc Natl Acad Sci USA* 1980, **77**, 4966–4970.
4. Van Laar JAM, Durrani FA, Rustum YM. Antitumor activity of the weekly intravenous push schedule of 5-fluoro-2′-deoxyuridine ±*N*-phosphonacetyl-L-aspartate in mice bearing advanced colon carcinoma 26. *Cancer Res* 1993, **53**, 1560–1564.
5. O'Dwyer PJ, Paul AR, Walczak J, Weiner LM, Litwin S, Comis RL. Phase II study of biochemical modulation of fluorouracil by low dose PALA in patients with colorectal cancer. *J Clin Oncol* 1990, **8**, 1497–1503.
6. Ardalan B, Singh G, Silberman H. A randomized phase I and II study of short time infusion of high dose fluorouracil with or without *N*-(phosphonacetyl)-L-aspartic acid in patients with advanced pancreatic and colorectal cancers. *J Clin Oncol* 1988, **6**, 1053–1058.
7. Grem JL, King SA, O'Dwyer PJ, Leyland-Jones B. Biochemistry and clinical activity of *N*-(phosphonacetyl)-L-aspartate: a review. *Cancer Res* 1988, **48**, 4441–4454.

8. Van der Wilt CL, Van Laar JAM, Gyergyay F, Smid K, Peters GJ. Biochemical modification of the toxicity and the antitumor effect of 5-fluorouracil and cis-platinum by WR-2721 in mice. *Eur J Cancer* 1992, **28A**, 2017–2024.
9. Voigt H, Kleeberg UR. PALA, Vindesine and cisplatin combination chemotherapy in advanced malignant melanoma. *Cancer* 1984, **53**, 2058–2062.
10. Kish JA, Enshley JF, Jacobs J, Weaver A, Cummings G, Al-Sarraf M. A randomized trial of cisplatin (CACP) + 5-fluorouracil (5-FU) infusion and CACP ± 5-FU bolus for recurrent and advanced squamous cell carcinoma of the head and neck. *Cancer* 1985, **56**, 2740–2744.
11. Al-Sarraf M. Clinical trials with fluorinated pyrimidines in patients with head and neck cancer. *Invest New Drugs* 1989, 7, 71–81.
12. Salter MM, Wheeler RH, Peters GE, *et al.* A phase I study of high-dose cisplatin prolonged infusion 5-fluorouracil, and concomitant conventional fraction radiation therapy in patients with inoperable squamous cell cancer of the head and neck. *Am J Clin Oncol* 1992, **15**, 461–463.
13. Pratt YZ, Boddie AW, Charnsangavej C, *et al.* Hepatic arterial infusion with floxuridine and cisplatin: overriding importance of antitumor effect versus degree of tumor burden as determinants of survival among patients with colorectal cancer. *J Clin Oncol* 1986, **4**, 1356–1364.
14. Esaki T, Nakano S, Tatsumoto T, *et al.* Inhibition by 5-fluorouracil of cis-disaminedichloro-platinum (II)-induced DNA interstrand cross-link removal in a HST-1 human squamous carcinoma cell line. *Cancer Res* 1992, **52**, 6501–6506.
15. Scanlon KJ, Newman EM, Lu Y, Priest DG. Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. *Proc Natl Acad Sci USA* 1986, **83**, 8923–8925.
16. Danhauser LL, Rustum YM. A method for continuous drug infusion in unrestrained rats: its application in evaluating the toxicity of 5-fluorouracil/thymidine combinations. *J Lab Clin Med* 1979, **93**, 1047–1053.
17. Corbett TH, Griswold DP Jr, Roberts BJ, Peckham J, Schabel FM Jr. A mouse colon-tumor model for experimental therapy. *Cancer Chemother Rep* 1975, **5**, 169–196.
18. Peters GJ, Van Dijk J, Nadal J, Van Groeningen CJ, Lanklema J, Pinedo HM. Diurnal variation in the therapeutic efficacy of 5-fluorouracil against murine colon cancer. *In Vivo* 1987, **1**, 113–118.
19. Van Laar JAM, Van der Wilt CL, Treskes MN, Van der Vijgh WJF, Peters GJ. Effect of WR-2721 on the toxicity and antitumor activity of the combination of carboplatin and 5-fluorouracil. *Cancer Chemother Pharmacol* 1992, **31**, 97–102.
20. Creaven PJ, Mayhew E, Pendyala L, *et al.* Cisplatin (C) modulation by PALA: a phase I and pharmacokinetic study. *Proc Am Soc Clin Oncol* 1994, **13**, 166.

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Influence of Indomethacin and Difluoromethylornithine on Human Tumour Growth in Nude Mice

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Biopsy material from six human colorectal carcinomas was transplanted to 114 nude mice. A treatment protocol was established which included no treatment (control, C), indomethacin (I), difluoromethylornithine (D) or a combination of both (ID). The influence of the various drugs on tumour weight and protein kinase CK2 activity was monitored. CK2 activity was measured because in all tumours examined so far the enzyme activity was found to be enhanced several-fold when compared to the non-neoplastic tissue of the same patient. More than half of the investigated tumours showed a conspicuous reduction in weight after drug treatment, and I and the combination of D/I were significantly effective using the mixed model analysis. Furthermore, we have tried to discover whether there is a change in the subcellular localisation of protein kinase CK2 subunits associated with drug treatment. We analysed the tumours and the non-neoplastic control tissues by immunohistochemistry using antibodies directed against the CK2 subunits and against the proliferation marker Mib. In addition, we have also investigated the behaviour of the nucleolar protein B23 which has also been shown to be enhanced several-fold in rapidly proliferating tissue and which is also a substrate for CK2. The immunohistochemical data suggest that, irrespective of the drug treatment and the observed reduction in CK2 activity, the CK2 subunits remain localised in the nucleus.

Key words: difluoromethylornithine, indomethacin, human colorectal carcinoma, nude mice, CK2
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