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Effect of WR-2721 on the toxicity and antitumor activity of the combination of carboplatin and 5-fluorouracil

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Summary. We evaluated the effects of WR-2721 on the toxicity and antitumor activity of the combination of 5-fluorouracil (5FU) and carboplatin (CBDCA) in BALB/c and C57Bl/6 mice. On a weekly schedule, i.p. injection of 200 mg/kg WR-2721 at 5 min prior to the administration of this combination enabled us to increase the CBDCA dose from a nontoxic level of 45 mg/kg to a normally toxic dose of 60 mg/kg in non-tumor-bearing BALB/c mice while maintaining the 5FU dose at 100 mg/kg. When WR-2721 was given 30 min before this combination, the CBDCA dose could not be increased to 60 mg/kg without producing drug-related deaths. WR-2721 protected against CBDCA- and 5-FU-induced thrombocytopenia but did not prevent leukopenia or anemia in C57Bl/6 mice. The antitumor activity of the combination against colon 26 tumors in BALB/c mice was increased by pretreatment with WR-2721, which facilitated elevation of the CBDCA dose to 60 mg/kg in combination with 100 mg/kg 5FU. These results reveal better therapeutic efficacy for the combination of 5FU and CBDCA following pretreatment with WR-2721.

Introduction

5-Fluorouracil (5FU) is currently used for the treatment of advanced colorectal cancer and for combination chemotherapy of head and neck cancer [1, 14]. 5FU has to be activated to the nucleotide level; 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) can inhibit thymidylate synthase (TS) in the presence of 5,10-methylene-tetrahydrofolate; 5-fluorouridine-5′-triphosphate (FUTP) can be incorporated into RNA; and 5-fluoro-2′-deoxyuridine-5′-triphosphate (FdUTP) can be incorporated into DNA and induce DNA strand breaks. Depending on the schedule, myelotoxicity is the dose-limiting factor of 5FU therapy [18].

The combination of 5FU and the DNA cross-linking cytostatic agent cisplatin (*cis*-diamminedichloroplatinum(II), CDDP) is used for the treatment of squamous-cell carcinoma of the head and neck [10]. The mechanism of action of this combination, which is thought to be at least additive, is unclear, although DNA double-strand breaks and a stabilizing effect of CDDP on the TS inhibited by 5FU have been suggested [13, 21].

Carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato) platinum(II), CBDCA) was developed as a second-generation analog of cisplatin that produces less nephro- and neurotoxicity than the parent drug. The dose-limiting toxicity of CBDCA in preclinical [22, 23] and clinical studies [3] has been myelosuppression along with severe thrombocytopenia and, less often, leukopenia. CBDCA has shown antitumor activity at least comparable with that of CDDP. Moreover, when given in combination with 5FU (and other cytostatics), CBDCA appears to be at least as potent as CDDP [30]. In patients with cancer of the head and neck, higher response rates have been obtained using the combination of CBDCA and 5FU as compared with CBDCA alone [6–8].

WR-2721 (S-2(3-aminopropylamino)ethylphosphorothioic acid, ethiofos), originally developed as a radioprotector [5, 19], can also protect against the dose-limiting side effects of anticancer agents. It has been shown to reduce adverse effects without diminishing the antitumor effect of chemotherapeutic agents such as CDDP and melphalan [9, 11, 31, 32]. This selectivity is thought to result from the preferential formation and uptake of the active thiol metabolite WR-1065 in normal tissues as compared with poorly vascularized, hypoxic tumor tissue [24]. Peak levels of WR-1065 in several nontumorous tissues have been attained within 5–30 min [28]. On the basis of the reaction kinetics of CBDCA with WR-2721, Treskes et al.

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Table 1. Effect of WR-2721 on the toxicity of 5FU and CBDCA

Dose (mg/kg)			MWL[b] (%)	Survival at day 42[c]
WR-2721[a]	5FU	CBDCA		
–	100	30	0.0– 6.5	2/3[d]
–	100	45	0.6– 7.9	3/3
–	100	60	2.4–11.2	3/6[d, e]
200/30	100	60	3.4–11.3	0/3[d, e]
200/ 5	100	60	2.2– 6.5	3/3
–	100	90	6.3–10.6	1/3[e]
200/30	100	90	4.8–13.1	0/3[e]
200/ 5	100	90	6.7–10.1	0/3[e]
200/ 5	100	180	6.0–17.3	0/3[e]

Drugs were given i.p. weekly for 4 weeks

[a] WR-2721 dose (mg/kg)/minutes prior to 5FU and CBDCA administration

[b] Maximal weight loss during the 1st week of treatment (ranges)

[c] Surviving mice/total number of mice

[d] Deaths occurred between day 21 and day 42

[e] Deaths occurred before day 21

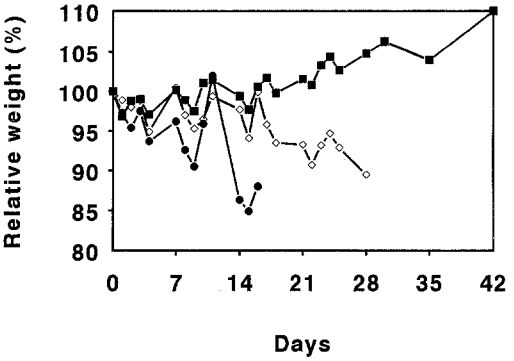

Fig. 1. Effect of variation of the interval between the administration of WR-2721 and that of CBDCA and 5FU on toxicity. WR-2721 given at 5 (-■-) or 30 (-●-) min prior to 5FU and CBDCA is compared with treatment with the combination in the absence of WR-2721 (-◇-). 5FU was given at 100 mg/kg; CBDCA, at 60 mg/kg; and WR-2721, at 200 mg/kg. Curves are plotted up to the median survival time (MST); the SD was generally less than 10%

[26] proposed that WR-2721 would have a better effect if it was given 5 instead of 30 min before CBDCA.

The present study was performed to investigate the cytotoxic side effects produced by the combination of 5FU and CBDCA in mice, the protection provided by WR-2721, and the antitumor effect of the triple combination on murine colon 26 tumors.

Materials and methods

Drugs. Formulated WR-2721 (ethiofos; 500 mg/vial) was obtained from U.S. Bioscience (West Conshohocken, Pa., USA) and was diluted with sterilized and pyrogen-free phosphate-buffered saline (PBS) to a concentration of 50 mg/ml. 5FU was formulated as a 50-mg/ml solution; this stock solution was diluted with sterilized and pyrogen-free PBS to a final concentration of 10 mg/ml. CBDCA (Paraplatin) was obtained from Bristol-Myers-Squibb (Woerden, The Netherlands) and was used at a concentration of 10 mg/ml.

Mice. Female BALB/c and C57Bl/6 mice aged 6 weeks were obtained from Harlan/Olac (Zeist, The Netherlands). The mice were kept in an area maintained on a standardized light-dark cycle and had access to food and water ad libitum. The initial estimation of the toxicity of the treatment was done using 3–6 mice/group to determine the safe doses for the combination therapy to be used in the subsequent experiments. Maximal weight loss during the 1st week was evaluated to determine the acute toxicity of the therapy. The maximal tolerated dose (MTD) was defined as that resulting in more than 90% survival and less than 10% weight loss during the experiments.

Weight was determined on the 1st day of treatment (day 0) and was set at 100%. Mice were weighed at least four times a week starting on the 1st day after treatment. Drugs were given i.p. weekly for 4 weeks at about 1300 hours. The number of survivors were determined for each group at 3 weeks after the end of the last treatment. A dose of 200 mg/kg WR-2721 was given on the basis of that previously used in combination with CDDP and 5FU [17]. Since we considered 5FU to be the active agent and CBDCA to be the modulating agent, we used 5FU at its MTD (100 mg/kg) and varied the dose of CBDCA from 30 to 180 mg/kg. 5FU and CBDCA were given simultaneously. Hereafter, the doses of CBDCA and 5FU are defined by their subscripts, i.e., $5FU_{100}$ means 100 mg/kg 5FU and $CBDCA_{30}$ means 30 mg/kg CBDCA.

Hematological toxicity. Hematological toxicity was assessed by determining hematocrit values and performing leukocyte and thrombocyte counts as previously described [12, 16] and was evaluated in non-tumor-bearing C57Bl/6 mice. C57/Bl6 mice were used for these experiments due to previous observations that the general pattern of toxicity in the two strains was comparable and that historical data were available for comparison with the new data [12, 15–17]. In addition, C57/Bl6 mice could be sampled more easily than BALB/c mice. Blood samples (80–150 µl) were collected at 3 days prior to the initiation of treatment and then at 4 days after each therapy and were analyzed as described elsewhere [16, 17]. Drugs were given i.p. weekly for 2 weeks.

Antitumor activity. The origin and characteristics of the murine colon 26 carcinoma (undifferentiated carcinoma with local fibrosarcoma) have been described elsewhere [15]. Tumors were transplanted s.c. into both flanks in small fragments of 1–5 mm^3. Before the initiation of therapy, mice were randomized into groups consisting of six animals each, one group serving as a control and the other being subjected to treatment. Mice were treated by i.p. injection at about 1300 hours once a week for 4 weeks. Therapy started at 10 days after transplantation by which time the tumors had reached a size of 50–200 mm^3. Tumor volume was determined twice weekly by caliper measurement (length × width × height × 0.5) on the day of treatment and 3 days thereafter. To determine relative tumor volumes, reference was made to the value obtained on day 0, which was the 1st day of treatment. The following parameters were calculated as previously described [15, 16]: T/C (tumor volume of the treated group/tumor volume of the untreated group × 100%), GDF [growth delay factor = tumor doubling time (TD) of the treated group (TD_{tr}–$TD_{control}$)/$TD_{control}$], and increased life span [ILS = median life span (MLS) of the treated group/MLS of the untreated group × 100%].

When appropriate, Student's *t*-tests for unpaired and paired data were used for statistical evaluation of the results; significance was assumed at $P < 0.05$. Details are given below.

Results

Survival and weight loss

Initial studies were carried out in non-tumor-bearing mice to find the optimal doses of the combination therapy to be used in the antitumor experiments. The maximal dose of CBDCA that could be given to non-tumor-bearing mice in

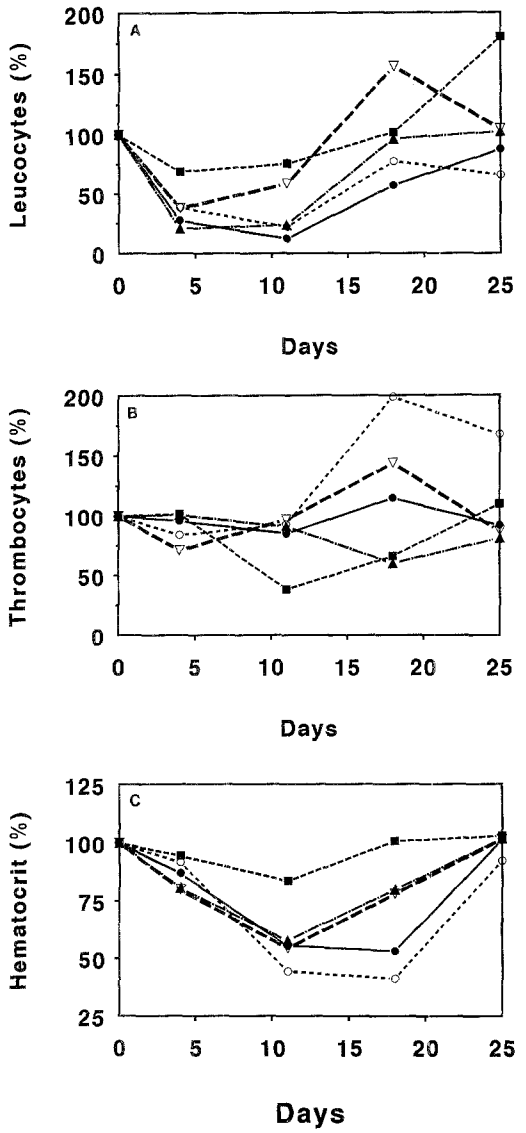

Fig. 2 A–C. Effect of WR-2721 on 5FU- and CBDCA-induced **A** leukopenia, **B** thrombocytopenia, and **C** anemia. WR-2721 was given 5 min before the combination. Data represent mean values for 4–6 mice and were calculated relative to those obtained before treatment in each mouse; the SD was less than 20%. Normal leukocyte and thrombocyte counts were $7.5 \pm 1.4 \times 10^6$ and $1053 \pm 223 \times 10^6$ cells/ml, respectively; mean hematocrit was 0.44 ± 0.02 ($n = 24$ mice). – – ○ – –, $5FU_{100}$; – ● –, WR-2721→5FU+$CBDCA_{60}$; – – ■ – –, $CBDCA_{60}$; – – ▽ – –, 5FU+$CBDCA_{30}$; – · – ▲ – · – –, 5FU+$CBDCA_{60}$. The degree of leukopenia caused by 5FU alone was significantly lower than that induced by $CBDCA_{60}$ alone ($P < 0.05$ on days 4 and 11) or by 5FU+$CBDCA_{30}$ ($P < 0.05$ on days 11 and 18), but it did not significantly differ from that caused by 5FU+$CBDCA_{60}$ or by WR-2721→5FU+$CBDCA_{60}$. The difference between WR-2721→5FU+$CBDCA_{60}$ and 5FU+$CBDCA_{60}$ was significant ($P < 0.01$). The degree of thrombocytopenia caused by 5FU+$CBDCA_{60}$ was significantly higher than that induced by WR-2721→5FU+$CBDCA_{60}$ ($P < 0.05$ on day 18). The rebound observed on day 18 for 5FU and for 5FU+$CBDCA_{30}$ differed significantly from that observed for 5FU+$CBDCA_{60}$ ($P < 0.01$), $CBDCA_{60}$ alone ($P < 0.01$), or WR-2721→5FU+$CBDCA_{60}$ ($P < 0.05$, but only as compared with 5FU alone). The anemia caused by all of the other treatments differed significantly from that induced by $CBDCA_{60}$ ($P < 0.01$ on days 11 and 18). On day 18, the anemia caused by 5FU and WR-2721→5FU+$CBDCA_{60}$ was significantly different from that induced by 5FU+$CBDCA_{60}$ or 5FU+$CBDCA_{30}$ ($P < 0.05$)

combination with $5FU_{100}$ was 45 mg/kg. All mice survived, and weight loss never exceeded 10% during the 1st week or the subsequent period (Table 1). Elevation of the CBDCA dose to 60 mg/kg in this combination was too toxic, although the weight loss did not exceed 10% during the 1st week (Fig. 1). The administration of WR-2721 at 30 min prior to the combination resulted in the death of all mice after four treatments (Table 1). However, when WR-2721 was given at 5 min prior to the treatment, we could safely increase the dose of CBDCA to 60 mg/kg while maintaining the dose of 5FU. All mice treated on this schedule survived, and the highest weight loss occurred on day 1. The high doses (90 and 180 mg/kg) of CBDCA given in combination with $5FU_{100}$ in the presence or absence of WR-2721 pretreatment (at both 5 and 30 min) were too toxic, resulting in considerable lethality (Table 1). However, one mouse survived that had received a high dose of CBDCA, but severe weight loss was observed. On the basis of these findings and those previously obtained using the combination WR-2721 and CBDCA [25], we used WR-2721 at 5 min prior to therapy for the hematological toxicity and antitumor activity experiments.

Hematological toxicity

A possible myeloprotective effect of WR-2721 (given 5 min before CBDCA and 5FU) was studied at the therapeutic dose of $5FU_{100}$ in combination with $CBDCA_{60}$. $CBDCA_{60}$ alone caused a slight decrease in leukocytes to 76% of pretreatment values, whereas $5FU_{100}$ alone caused severe leukopenia. $5FU_{100}$ given in combination with a lower CBDCA dose (30 mg/kg) caused a drop to only 58% of pretreatment levels, whereas $5FU_{100}$ used in combination with $CBDCA_{60}$ induced severe leukopenia. WR-2721 did not protect against 5FU–CBDCA-induced leukopenia, as the level of leukocytes decreased to as low as of normal values on day 11 (Fig. 2a).

$CBDCA_{60}$ alone resulted in a severe decrease in thrombocytes, whereas $5FU_{100}$ alone caused only an increase. $5FU_{100}$ and $CBDCA_{30}$ resulted in a slight decrease in thrombocytes followed by a rebound. The combination of $CBDCA_{60}$ and $5FU_{100}$ caused delayed thrombocytopenia as compared with the other schedules. However, pretreatment with WR-2721 prevented this nadir, and the thrombocyte profile resembled that obtained for the combination using a low dose of CBDCA (Fig. 2b). CBDCA alone produced a minimal reduction in the hematocrit, whereas 5FU alone caused anemia. On the other schedules, hematocrit values were slightly higher than those obtained using 5FU alone, with no significant difference being found (Fig. 2c).

Antitumor activity

We studied the antitumor activity of 5FU and CBDCA at three different CBDCA doses (Table 2) on the basis of the results of the MTD experiments. The MTD for the combination of these two drugs was $5FU_{100}$ and $CBDCA_{45}$ in non-tumor-bearing mice. On the basis of our previous ex-

Table 2. Antitumor activity of 5FU and CBDCA in combination with WR-2721

Schedule and dose[a]	T/C[c] (%)	ILS[d] (%)	MWL[e] (%)	GDF[f]
5FU	41	307	4.8±1.9	3.4
$CBDCA_{90}$	52	343	12.2±9.6	1.0
$5FU+CBDCA_{30}$	43	307	5.4±1.7	5.8
$WR^{b}\rightarrow 5FU+CBDCA_{60}$	22	430	7.0±4.0	9.2

[a] The dose of 5FU was 100 mg/kg and the doses of CBDCA are defined by their subscripts in mg/kg. Therapy was given weekly for 4 weeks
[b] WR-2721 was given i. p. 5 min prior to CBDCA therapy
[c] Maximal T/C values
[d] Increase in life span
[e] Maximal weight loss during the 1st week of treatment (mean values ± SD)
[f] Tumor growth-delay factor

The antitumor effect of the WR-2721→5FU+$CBDCA_{60}$ combination was significantly greater than that of 5FU+$CBDCA_{30}$ (P <0.05), 5FU alone (P <0.01), or $CBDCA_{90}$ alone (P <0.01). Monotherapy with $CBDCA_{90}$ was significantly less effective than treatment with 5FU alone (P <0.05) or with 5FU+CBDCA

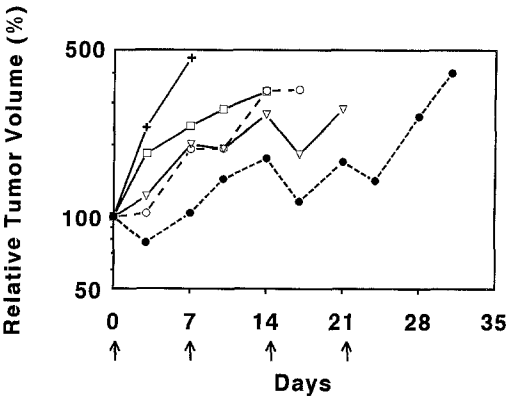

Fig. 4. Antitumor activity of WR-2721, 5FU, and CBDCA in female BALB/c mice bearing colon 26 tumors. WR-2721 was given 5 min prior to the combination. Data represent mean values (n = 6 mice) calculated relative to those obtained on day 0. Curves are plotted up to the MST; the SD was generally less than 40%. Statistical significance is discussed in Results. – + –, Controls; – – ○ – –, $5FU_{100}$; – □ –, $CBDCA_{90}$; – – ● – –, WR-2721→5FU+$CBDCA_{60}$, -▽-, 5FU+$CBDCA_{30}$

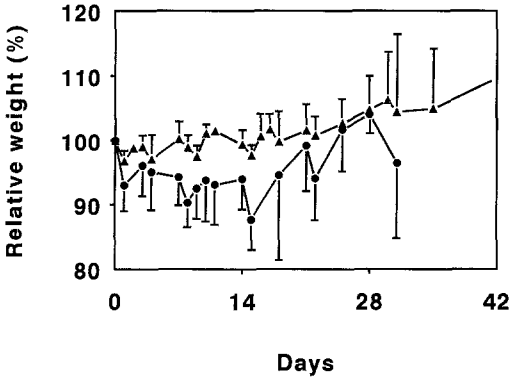

Fig. 3. Comparison of weight loss by tumor bearing (– ● –) and non-tumor-bearing (– ▲ –) female BALB/c mice. WR-2721 was given 5 min prior to the administration of 100 mg/kg 5FU and 60 mg/kg CBDCA. Curves are plotted up to the MST; data represent mean values ± SD for 3–6 mice

perience with CDDP and 5FU [17], we decreased the dose of CBDCA to 30 mg/kg in tumor-bearing animals. Tumor-bearing mice tolerated less CBDCA in combination with 5FU than did non-tumor-bearing mice (Fig. 3). Treatment with $CBDCA_{90}$ alone increased the life span considerably in comparison with that of nontreated animals, but the antitumor effect was moderate (difference in tumor volumes versus control values, P <0.05) and considerable weight loss was observed (12.2% on day 7). The weight loss was not attributable to a reduction in the size of the tumor since the total tumor size never exceeded 0.5 g and $CBDCA_{90}$ caused only a delay in tumor growth but no reduction in size. $5FU_{100}$ alone produced results comparable with those obtained in earlier experiments [17]. Its combination with $CBDCA_{30}$ did not improve its antitumor activity significantly during the first 2 weeks, but thereafter, a significant difference in tumor volumes (P <0.001) was observed for the combination as compared with 5FU alone. Elevation of the CBDCA dose to 60 mg/kg in combination with $5FU_{100}$ following WR-2721 pretreatment resulted in an improved antitumor effect as compared with the tumor volume obtained using $5FU_{100}$+$CBDCA_{30}$ ($0.001<P<0.01$; Table 2, Fig. 4). Whether WR-2721 might have protected tumor tissue against CBDCA could not be studied; by adding 30 mg/kg CBDCA to $5FU_{100}$, we achieved a slight potentiation of the antitumor activity of 5FU alone. A possible reversal of the additional effect of $CBDCA_{30}$ by WR-2721 would not have been detectable (WR-2721 does not interact with 5FU). Furthermore, higher doses of CBDCA (45 or 60 mg/kg) combined with FU_{100} in tumor-bearing mice could not be given without WR-2721 because of lethal toxicity, thus unfortunately preventing a direct and evaluable comparison of these schedules in the presence and absence of WR-2721.

Discussion

Our results demonstrated that WR-2721 protected mice against CBDCA-induced weight loss, mortality, and thrombocytopenia when CBDCA was combined with 5FU. The optimal protective effect was obtained when WR-2721 was given 5 min prior to the combination and was similar to the protective effect of WR-2721 against CDDP-induced toxicity [27]. Protection against leukopenia or anemia was not observed. The overall modulating effects of WR-2721 enabled us to increase the dose of CBDCA, which resulted in a better antitumor effect against colon 26 tumors for the combination of CBDCA with 5FU.

Dephosphorylation of WR-2721 and subsequent uptake of WR-1065 occurs within minutes [24], and interference with CBDCA in the bloodstream is limited [26]. These findings and the initial MTD studies suggested that scheduling of WR-2721 was an important factor. Optimal protection was obtained when WR-2721 was given at 5 instead of 30 min prior to CBDCA administration, which is the most commonly used schedule based on radiotherapy

studies [9]. Whether tumor protection by WR-2721 occurred could not be determined, but on the plasma level, WR-2721 does not interact with CBDCA [26]. In a xenograft of human ovarian cancer, we observed that WR-2721 did not decrease the antitumor effect of $CBDCA_{60}$, but slightly enhanced it [25]. It should be noted that at the 30-min interval, the combination of WR-2721 with 5FU and CBDCA was more toxic than the two drugs alone. The reason for this finding cannot be deduced from these experiments, but it does stress the importance of proper scheduling. When given at an interval of 5 min prior to $CBDCA_{90}$ administration to mice, WR-2721 provided better protection against the reduction of in vitro proliferation of whole bone-marrow cells than that observed following its administration 30 min prior to or after $CBDCA_{90}$ treatment [25]. This phenomenon is probably related to the slow interaction of CBDCA and WR-2721 [25].

The nephrotoxicity and gastrointestinal (GI) toxicity induced by CBDCA are lower than those induced by CDDP. In phase I and II trials of combinations of 5FU and CBDCA, myelosuppression was dose-limiting [8]. We observed that thrombocytopenia caused by the combination of CBDCA and 5FU can be prevented by WR-2721, whereas leukopenia and anemia cannot. Thus, WR-2721 apparently protects not at the level of the totipotential hemopoietic stem cells but in cells committed at the megakaryocytic level. This might be of clinical importance, as the dose-limiting toxicity of CBDCA mostly manifests as thrombocytopenia. Leukopenia and anemia are primarily 5FU-induced effects that cannot be ameliorated by WR-2721. The lack of increased anemia observed for the combination of CBDCA and 5FU may have been related to the relatively low dose of CBDCA used. The weight loss of non-tumor-bearing mice in the 1st week might have been a result of GI toxicity, since mucosal damage can occur very rapidly. Weight loss induced by myelosuppression usually occurs after 10–14 days. Our results indicate that WR-2721 could protect mice against immediate GI toxicity, since minimal weight loss was observed during the 1st week in the group pretreated with WR-2721. Millar et al. [11] demonstrated that WR-2721 could protect mice against immediate GI toxicity induced by melphalan. Other distinctive protective effects of WR-2721 have not been investigated, but it apparently facilitates the administration of a higher CBDCA dose in combination with 5FU by preventing CBDCA-induced weight loss and toxic death.

We did not try to elevate the 5FU dose in this combination, since WR-2721 did not protect mice against 5FU-induced side effects [17]. The 5FU dose was maintained at 100 mg/kg, which was the MTD, and 5FU was considered to be the active drug of this combination in the treatment of GI tumors. We expected CBDCA to potentiate 5FU antitumor activity. CBDCA has shown antitumor activity at least comparable with that of CDDP in preclinical [20] and clinical studies [2]. Combination therapy with CBDCA and 5FU has provided better induction chemotherapy in the treatment of head and neck cancers as compared with CDDP and 5FU due to the similar antitumor effect and the lower toxicity of the former regimen [29]. The additional effect of CBDCA in this combination seemed to be dose-dependent because the optimal antitumor activity was increased when the CBDCA dose was escalated and the 5FU dose was maintained at 100 mg/kg. The addition of 30 mg/kg CBDCA to $5FU_{100}$ (the MD of the combination in tumor-bearing mice in the absence of WR-2721 pretreatment) moderately improved the antitumor activity of 5FU alone. $CBDCA_{90}$ alone caused an increase in life span and had a slight antitumor effect. CBDCA used alone at a dose lower than 90 mg/kg is not likely to be as effective as $CBDCA_{90}$. Thus, we assume that WR-2721 pretreatment facilitated the action of CBDCA to potentiate the effect of the relatively inactive 5FU. The mechanism of the 5FU-CBDCA combination has not yet been determined, but because CBDCA's action is similar to that of CDDP, it might act comparably with CDDP when used in combination with 5FU. For the latter combination both synergistic and additive effects have been described. Although stabilization of TS inhibition by the platinum compound has been proposed on the basis of a decrease in FdUMP binding [13, 21], we recently obtained evidence that inhibition of dUMP conversion to TMP is not enhanced in the combination of 5FU, CDDP, and WR-2721 (Van der Wilt et al., submitted for publication). Induction of DNA strand breaks might be of more importance in the combination of either CDDP or CBDCA with 5FU. Cohen and Robins [4] have reported that the antitumor activity of CBDCA is increased by hyperthermia. We have previously described a decrease in temperature in mice after the administration of WR-2721 [17], and hypothermia (>7° C) was also observed in our latest experiments using WR-2721 (data not shown). On the basis of these findings and assuming that a decrease in temperature might lower the antitumor activity of CBDCA, it would seem that WR-2721 might reduce this antitumor activity. However, the increased antitumor effect observed in the present study indicates at least an additive effect for CBDCA in the regimen consisting of CBCDA and 5FU.

It can be concluded that WR-2721 protects mice against CBDCA-induced toxicity in the combination of CBDCA with 5FU. In chemotherapeutic regimens against cancer of the head and the neck, CBDCA plus 5FU have increased the response rates obtained [7, 8]. WR-2721 offers protection against CBDCA-induced toxicity [27] and might increase the response rates by enabling the use of higher CBDCA doses in this combination. A therapeutic benefit may be gained in the treatment of squamous carcinoma of the head and neck and, possibly, other tumors by the inclusion of WR-2721 in a 5FU- and CBDCA-containing regimen so as to increase the dose of the latter compound.

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