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Response of Nude Mouse-grown Human Urothelial Cancer to *cis*-Diammine-dichloroplatinum(II), Diammine[1,1-cyclobutanedicarboxylato(2-)-*O*,*O*′-platinum], and Mitoguazone Dihydrochloride[1, 2]

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ABSTRACT

A comparative study of the effect of *cis*-diamminedichloroplatinum(II) (cisplatin), diammine[1,1-cyclobutanedicarboxylato(2-)-*O*,*O*′-platinum] (carboplatin), and mitoguazone dihydrochloride on urothelial cancer was conducted using transitional cell carcinomas of the urinary bladder grown in the nude mouse. Tumors SW-780 and TCC-K1 represented transitional cell carcinoma, Grade II, whereas Tumor PR49 represented a fast-growing Grade III neoplasm. Of the agents studied, cisplatin was most effective, resulting in tumor response related to the dose administered. Response to carboplatin was clearly related to treatment schedule. For the same amount of total dose administered, better results were obtained when treatment was given three times weekly instead of once every week. Furthermore, cisplatin was more effective against the less differentiated PR49 tumor in contrast to carboplatin, which showed more activity against the better differentiated SW-780 and TCC-K1 tumors. None of the tumors tested responded to mitoguazone dihydrochloride. The results of the present study may assist in formulating better treatment modalities against urothelial cancer, taking into account factors such as tumor grade, growth rate, treatment schedule, and the patient's tolerance which may ultimately influence tumor response.

INTRODUCTION

TTC[4] of the urinary bladder is an important disease with an annual incidence in the United States of approximately 35,000 (1). Invasive bladder cancer carries a high mortality, accounting for approximately 10,000 deaths annually (1) despite the fact that a number of treatment modalities have been used, including surgery, radiation, chemotherapy, and/or combinations of these procedures. Treating bladder cancer becomes a challenge, considering the fact that even superficial tumors have a 50 to 77% rate of recurrence, even after a seemingly successful treatment (2–4).

In a number of recent publications, various therapeutic modalities used in treating bladder cancer have been discussed (5–7). The results of these studies show that a small number of cytotoxic agents used singly and in various combinations have a beneficial effect, resulting in partial tumor remission of a few months duration. This indicates that urothelial cancer is a chemotherapy-responsive tumor (7) and emphasizes the need for additional studies to evaluate old and mainly new and untested cytotoxic agents at both the experimental and clinical levels.

During the past years, we have been using the athymic nude mouse to grow TCC of the bladder in an effort to study their response to various treatment modalities. More recently, we have completed a comparative study of the response of nude mouse-grown TCC to a number of cytotoxic agents. The efficacy of cisplatin, carboplatin, and MGBG on TCC was studied, and tumor responses for each drug tested were compared and evaluated. This paper reports on the results of these studies.

MATERIALS AND METHODS

Female BALB/c athymic nude mice, 4 to 6 weeks old, obtained from the Charles River Breeding Laboratory, Wilmington, MA, were used throughout.

For this study, we selected 3 TCC of the urinary bladder grown in the nude mouse. Tumor PR49, TCC Grade III, originated from a 65-year-old Caucasian male; Tumor SW-780, TCC Grade II, originated from an 81-year-old Caucasian female; and Tumor TCC-K1, Grade II, originated from a 64-year-old Caucasian male. None of the donor patients had any chemotherapy treatment prior to tumor establishment in the nude mouse. The biological characteristics of these tumors in the nude mouse have been reported recently (8, 9). For this study, mice received s.c., through a skin incision in the anterior aspect of the lateral thoracic wall, a small piece of tumor measuring approximately 0.2 to 0.3 cm (10). Treatment was initiated when tumors had reached a measurable size of 100 to 150 mm cu. Tumor-bearing mice were randomized to groups of 6 animals each, including the untreated control groups. Cisplatin, unless otherwise stated, was administered in the amount of 5 mg/kg once a week or in the amount of 2.5 mg/kg twice weekly; both treatment schedules were given for 4 consecutive weeks. Preliminary experiments were carried out to determine the acute toxicity of carboplatin[5] in tumor-bearing mice. Initially, carboplatin was given in the amount of 75 mg/kg once weekly and 25 mg/kg 3 times weekly. Both treatment schedules resulted in acute toxic deaths of 3 of 6 animals (50%) following the second weekly treatment. Therefore, in the final experimental study, carboplatin was given in the amount of 60 mg/kg once weekly for 4 weeks and 20 mg/kg 3 times a week for 4 weeks, both groups receiving the same total amount of carboplatin. MGBG was administered in the amount of 50 mg/kg 3 times per week for a total of 4 weeks. All treatments were given i.p. From the initiation to the termination of the experimental studies, all animals, treated and control, were weighed, and tumor volumes, expressed in cu mm, were calculated using the formula

$$L \times W^2 \times 0.4$$

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[2] This work is dedicated to the memory of Dr. Jørgen Fogh, who passed away on December 28, 1984.

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[4] The abbreviations used are: TCC, transitional cell carcinoma; MGBG, mitoguazone dihydrochloride; cisplatin, *cis*-diamminedichloroplatinum(II); carboplatin, diammine[1,1-cyclobutanedicarboxylato(2-)-*O*,*O*′-platinum].

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[5] Carboplatin was obtained from the Pharmaceutical Resources Branch, National Cancer Institute.

(11) where *L* is the larger tumor diameter, and *W* is the shorter tumor diameter. At the end of the experimental period, animals were killed by cervical dislocation, and a complete autopsy was performed.

Statistics. Tumor response to various treatments was evaluated by the Student *t* test.

RESULTS

In all 3 tumors studied, there was no difference in treatment-related tumor response between 5 mg of cisplatin per kg given once weekly and 2.5 mg/kg given twice weekly, both groups receiving the same total amount of cisplatin. Therefore, only the groups receiving 5 mg of cisplatin per kg are presented for discussion.

Tumor SW-780. Tumor SW-780 showed a marked sensitivity to cisplatin treatment. Arrest of tumor growth was evident immediately after initiation of treatment, and tumor regression was seen by the third treatment week. By the seventh week, tumors had completely regressed, and all animals were tumor free for a 3-week period (seventh to ninth weeks). However, by the tenth week, 2 of the tumors reappeared. A third tumor recurred by the 13th week, and a fourth one started growing by the 16th week. Two of the treated animals remained free of tumor, and at the time of their sacrifice, 6 months posttreatment, no tumor was present at the site of tumor implantation (Chart 1).

Response to carboplatin treatment depended largely on the treatment schedule. Tumors treated with 60 mg/kg, the treatment given once weekly, continued to grow for the first 5 weeks at a rate similar to that of the control group. By the fifth week, arrest of tumor growth became apparent, lasting for 5 weeks. Following this period, an accelerated tumor growth resulted in tumor size comparable to that of the control group within a 2-week period (Chart 1). When carboplatin was administered at a rate of 3 times per week, 20 mg/kg/injection, tumor growth arrest was observed by the second week, lasting for 7 weeks. This was followed by a period of slow, gradual tumor regrowth, reaching the size of the control group 19 weeks posttreatment. A comparison of the 2 carboplatin treatments showed a statistically significant difference in growth rate between the 2 groups which persisted throughout the entire experimental period (Chart 1; Table 1).

MGBG had no influence on tumor growth, the growth curves of both treated and control animals being similar (Chart 1; Table 1). Table 1 summarizes the statistical evaluation of tumor response following various treatments.

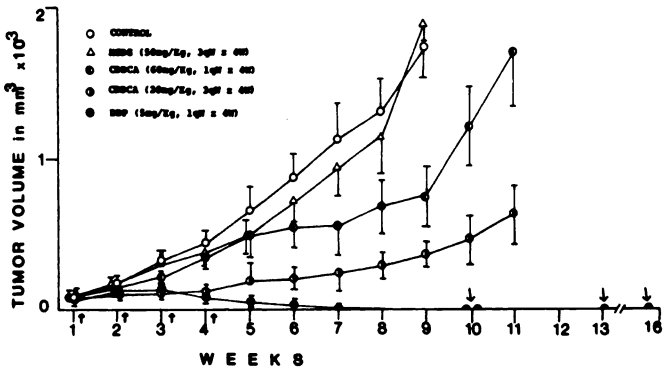

Chart 1. Growth curves of Tumor SW-780 following various treatments. ↓, time of reappearance of tumor after a period of almost complete regression; ↑, weeks of treatments. *CBDCA*, carboplatin; *DDP*, cisplatin. ○, control; △, MGBG (50 mg/kg, 3qW × 4W); ◐, CBDCA (60 mg/kg, 1qW × 4W); ◑, CBDCA (20 mg/kg, 3qW × 4W); ●, DDP (5 mg/kg, 1qW × 4W). *3qW × 4W*, 3 times weekly for 4 weeks; *1qW × 4W*, once per week for 4 weeks. *Points*, mean of 6 animals; *bars*, SD.

Tumor TCC-K1. Cisplatin treatment resulted in tumor growth delay evidenced immediately following initiation of treatment. The difference in growth rates between the treated and the control groups became apparent as early as the second week and persisted throughout the entire experimental period. Measurements taken at weekly intervals showed a statistically significant difference at all points of measurements taken during the active tumor growth (Chart 2).

Carboplatin, given once weekly in the amount of 60 mg/kg, had no effect on tumor growth (Chart 2). However, carboplatin treatment administered 3 times per week at 20 mg/kg/injection resulted in tumor response comparable to that observed following cisplatin treatment. Beyond the ninth week, however, carboplatin-treated tumors showed a much slower growth rate when compared with that seen in the cisplatin-treated group (Chart 2).

Tumor exposed to MGBG showed no response to treatment. Table 1 summarizes the statistical evaluation of tumor response following various treatments.

Tumor PR49. Tumor PR49 was found to be extremely sensitive to cisplatin. Tumor response became apparent immediately after initiation of the treatment. By the fourth week, all tumors had completely regressed. The animals of this group were kept under observation for 6 months. There was no tumor recurrence during that period, and at autopsy, at the termination of the experiment, no evidence of residual tumor was seen at the site of tumor implantation and growth (Chart 3). In order to evaluate the dose-dependent tumor response, a group of animals bearing Tumor PR49 received 3 mg of cisplatin per kg once weekly for 4 weeks.[6] Tumor response to that treatment became evident following the second treatment week and reached maximum response with a tumor size of 238 cu mm by the sixth week. This, however, was followed by a period of rapid tumor regrowth with treated tumors reaching the size of the control group by the tenth week, showing a 3-week growth delay period (Chart 3).

Tumor response to carboplatin was dependent to a certain degree on the treatment schedule. No tumor response was observed when carboplatin was given once weekly. However, fractionation of the same amount of carboplatin into 3 weekly injections resulted in a brief period of slower tumor growth evident during the fifth, sixth, and seventh weeks. These tumors reached the size of the control group by the tenth week (Chart 3).

Treatment with MGBG was ineffective in controlling tumor growth. Table 1 summarizes the statistical evaluation of tumor response to various treatments.

During the experimental period, the animals tolerated well the treatments without evidence of acute toxicity which would have been reflected in marked changes in animal body weight and acute or delayed toxicity deaths (Chart 4).

DISCUSSION

The data presented indicated that all 3 TCCs responded to cisplatin treatment, although with variable intensity. Of the 3

[6] Tumors SW-780 and TCC-K1 were not tested at this dose level, since previous dose-response studies had shown that both tumors were refractory to this treatment schedule. A. P. Kyriazis, unpublished observations.

Table 1

Statistical evaluation of tumor response to cisplatin, MGBG, and carboplatin

Evaluation was performed at the time of termination of controls.

Tumor line	Control vs. MGBG			Control vs. DDP[a]			Control vs. CBDCA 1[b]			Control vs. CBDCA 2[c]			CBDCA 1 vs. CBDCA 2		
	$X_i - X_{ii}$[d]	% of change	*P*	$X_i - X_{ii}$	% of change	*P*	$X_i - X_{ii}$	% of change	*P*	$X_i - X_{ii}$	% of change	*P*	$X_i - X_{ii}$	% of change	*P*
SW-780	−161	−9	NS	—[e]	100	<0.001	1000	57	<0.001	1282	73	<0.001	281	37	=0.05
TCC-K1	−82	−3	NS	1572	61	<0.001	446	17	NS	1839	71	<0.001	1393	65	<0.001
PR49	86	2.5	NS	—	100	<0.001	19	0.6	NS	1335	42	<0.001	1316	42	<0.001

[a] DDP, cisplatin; CBDCA, carboplatin; NS, statistically not significant.
[b] CBDCA 1, 60 mg/kg, once weekly for 4 weeks.
[c] CBDCA 2, 20 mg/kg, 3 times per week for 4 weeks.
[d] Difference between means of compared groups, 6 animals/group.
[e] —, complete tumor regression at the time of evaluation.

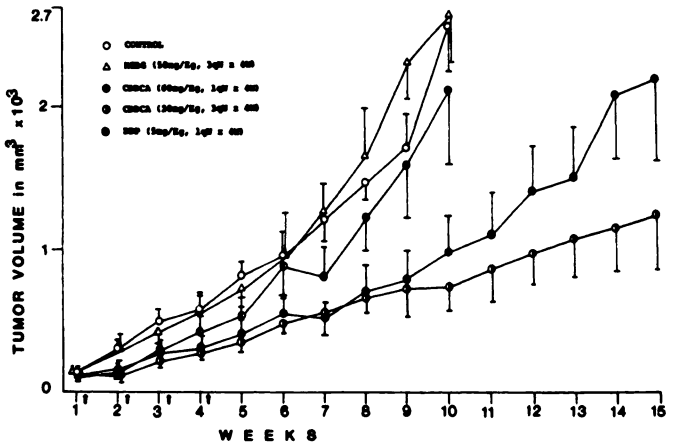

Chart 2. Growth curves of Tumor TCC-K1 following various treatments. *Points*, mean of 6 animals; *bars*, SD. ↑, weeks of treatment. ○, control; △, MGBG (50 mg/kg, 3qW × 4W); ◐, CBDCA (60 mg/kg, 1qW × 4W); ◑, CBDCA (20 mg/kg, 3qW × 4W); ●, DDP (5 mg/kg, 1qW × 4W). Abbreviations are as defined in the legend to Chart 1.

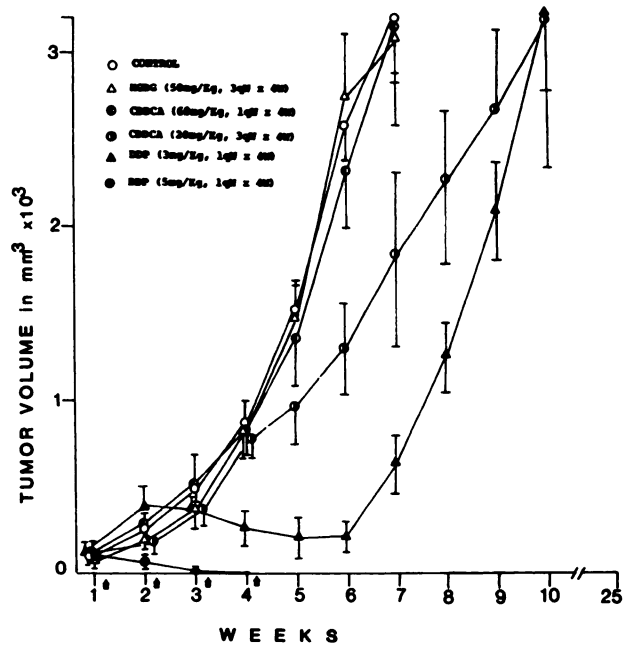

Chart 3. Growth curves of Tumor PR-49. *Points*, mean of 6 animals; *bars*, SD. ↑, weeks of treatment. ○, control; △, MGBG (50 mg/kg, 3qW × 4W); ◐, CBDCA (60 mg/kg, 1qW × 4W); ◑, CBDCA (20 mg/kg, 3qW × 4W); ▲, DDP (3 mg/kg, 1qW × 4W); ●, DDP (5 mg/kg, 1qW × 4W). Abbreviations are as defined in the legend to Chart 1.

tumors studied, PR49, a poorly differentiated fast-growing TCC (8), showed the best response with complete tumor regression. This was in contrast to the other 2 TCCs in which tumor recurrence was a consistent finding. Whether the degree of tumor response to cisplatin is related to tumor grade and growth rate, as our data seemed to suggest, remains a question requiring further studies. An additional observation was that better tumor response was achieved when mice were exposed to cisplatin at the upper limits of tolerance as was clearly demonstrated in the case of Tumor PR49 (Chart 3). Furthermore, tumor response, for the same amount of cisplatin, was unrelated to treatment schedule, an observation further corroborating our previous observations (12).

Carboplatin, given in the amount of 60 mg/kg in a single weekly treatment, had no effect on Tumors PR49 and TCC-K1 with only a brief transient response seen with Tumor SW-780. However, fractionated administration of the same amount of carboplatin resulted in considerable tumor response for all 3 tumors studied. Under this schedule, Tumor TCC-K1 showed a response comparable to that of cisplatin (Chart 2).Tumor SW-780 showed a response which, when compared with that seen in the single weekly treatment, was significantly different (Table 1); and Tumor PR49 showed a significant, although brief, growth delay for a 3-week period following termination of the treatment. These observations indicated that tumor response to carboplatin was schedule dependent, as contrasted to the efficacy of cisplatin, which was primarily dose dependent and unrelated to treatment schedule. These results are compatible with the available information on the pharmacokinetics of cisplatin and carboplatin in various species. It is well documented that cisplatin concentration in the serum shows an initial half-life of up to 1 h, followed by a terminal half-life of several days duration, during which a tissue plateau concentration is reached, lasting for several days (13–20). In contrast, carboplatin, slowly bound to plasma proteins, is excreted in the urine the first 24 h after administration (21–25). It is reasonable to assume that fractionated administration of carboplatin results in a relatively stable tissue concentration, resulting in an enhanced therapeutic effect.

Growth curve characteristics presented in Charts 1 and 2 showed that, where marked tumor response was apparent, with increasing antitumor effect, there was a reduced slope for regrowing tumors. We have observed this tumor regrowth behavior with other tumor systems and under different treatments. Whether this represents a tumor bed effect or enhanced immunity subsequent to cytoreductive therapy is a matter of specu-

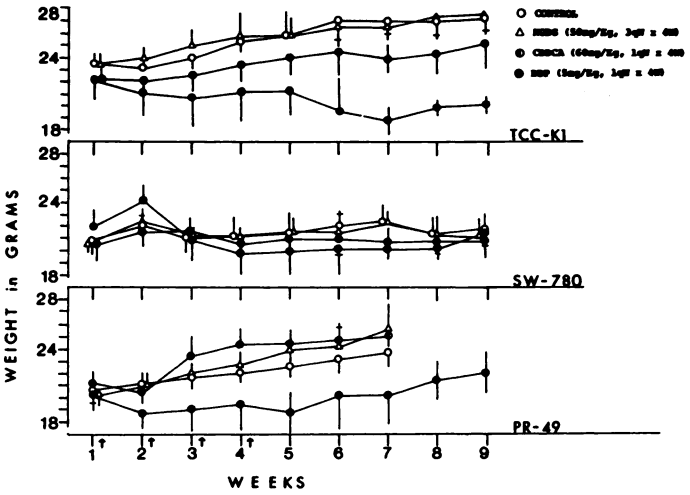

Chart 4. Animal body weight following various treatments. Of the 3 agents studied, only cisplatin treatment affected animal body weight during the treatment period. Body weight of animals receiving weekly treatment did not differ from that of animals receiving fractionated cisplatin and carboplatin treatment (not shown in the chart). ○, control; △, MGBG (50 mg/kg, 3qW × 4W); ◐, CBDCA (60 mg/kg, 1qW × 4W); ●, DDP (5 mg/kg, 1qW × 4W). *Points*, mean of 6 animals; *bars*, SD. Abbreviations are as defined in the legend to Chart 1.

lation. Furthermore, a successful treatment would eliminate all sensitive cells, leaving only treatment-resistant tumor cells. The proliferative capacity of the latter, however, may have also been affected by the treatment, resulting in a slower rate of cell turnover. Thus, a reduced slope for regrowing tumors may be the expression of a posttreatment recovery period.

In conclusion, our experiments have indicated that, of the 3 antineoplastic agents investigated, cisplatin was the most effective single agent against urothelial cancer, the response determined primarily by the dose administered. On the other hand, carboplatin, a second-generation platinum analogue, demonstrated antitumor activity which, in contrast to cisplatin, was dependent on the treatment schedule. An additional observation was that carboplatin was most effective against the better-differentiated SW-780 and TCC-K1 tumors, whereas cisplatin was extremely effective against the less-differentiated PR49 TCC. It is hoped that the information presented may assist in the formulation of more effective treatment of urothelial cancer, taking into account such factors as treatment schedule and tumor grade.

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