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Experimental studies of chemosensitivity testing of urothelial cancer grown in nude mice

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Summary. The present study provides information relevant to a number of variables which may influence response to treatment of nude mouse-grown human urothelial cancer. A number of xenotransplanted tumors were exposed to selected treatments at different transplant generations, and at various dose levels and treatment schedules. It was observed that nude mouse-grown tumors were characterized by consistency, reproducibility and biological stability not affected by the transplant generation at which they were examined. Treatment related dose response curves were steep, the sharpness of the curves depending on the degree of tumor sensitivity. Best therapeutic results were obtained at the maximum tolerated dose of cytotoxic agents under study and of importance, a 20% to 40% dose reduction with the same treatment schedule resulted in little or no activity. In addition, treatment schedule, timing and sequence of treatments and to a certain degree, tumor grade were important variables which could influence tumor response. The nude mouse-human tumor system provides important preclinical guidelines on dose, schedule, sequence and timing of treatments and can assist in designing more efficient clinical trials.

Key words: Urothelial cancer – Nude mouse – Xenotransplanted urothelial cancer – Experimental cancer therapeutics

The nude mouse, due to the lack of cell mediated immunity, provides an ideal host for the development of transplantable human tumors representing a wide range of histiotypic diversity. Since the first report of a human tumor line established in the nude mouse [22], a number of laboratories, including ours, have used the nude mouse-human tumor system in studies of various facets of tumor biology and in cancer treatment research. The results of many years of research have indicated that nude mouse-grown human tumors mimic the phenotypic and genotypic characteristics of the tumor of origin [6, 9, 10, 11, 13, 20], and retrospective analysis of the information available from clinical studies have indicated that xenotransplanted human tumors show responses mimicking those expected from tumors of the same type in the human.

Most studies, however, represent isolated efforts and there is an obvious paucity between the information generated and the present state of our knowledge as to the clinical significance of available data as it becomes available. Furthermore, since nude mouse-grown human tumors are propagated through serial transplant generations no information exists as to the biological stability of such tumors and no attempt has been made to explore variables which may affect tumor response to treatment. This report attempts to answer some of these questions.

Material and methods

For these studies nude mouse-established transitional cell carcinomas of the urinary bladder (TCC) were used. The biological characteristics of these tumors and their response to selected treatments have been reported [12, 14, 15, 16, 17]. None of the donor patients had received any treatment prior to tumor establishment in the nude mouse. Female BALB/c athymic mice, 4 to 6 weeks old, were obtained from the Charles River Breeding Laboratory, Wilmington, MA. Mice received subcutaneously, through a skin incision in the anterior aspect of the lateral thoracic wall, a small piece of tumor measuring approximately 0.2×0.3 cm [7].

Treatment was initiated when tumors had reached a measurable size of 100 to 150 cu mm. Tumor-bearing mice were randomized to groups of 6 animals each, including the untreated control groups. All therapy was administered intraperitoneally. Cytotoxic agents with the dosages and treatment schedules employed are detailed in the text. Mice, treated and control, were weighed and tumor volumes were measured weekly with calipers using the formula length $\times$ (width)$^2 \times$ 0.4. Treatment evaluation was based on tumor volume analysis and statistical evaluation was performed using the Student's t test.

Results

We have investigated the growth behavior of a number of nude mouse-established human bladder transitional cell

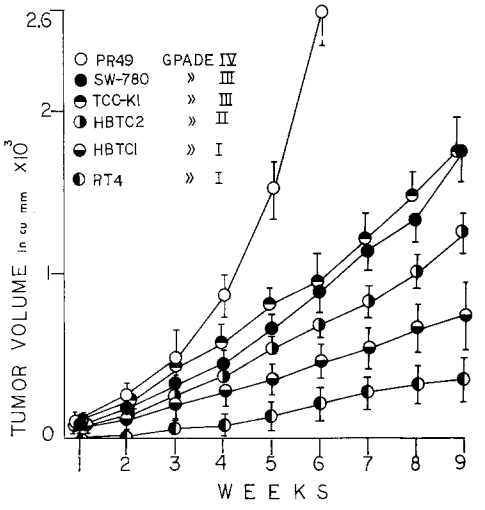

Fig. 1. Growth curves of 6 TCC grown s.c. in the nude mouse. Tumor PR49, a poorly differentiated TCC showed the fastest growth rate, whereas tumors RT4 and HBTC1, both well differentiated TCC, were characterized by a much slower growth rate. Tumors of grades II and III exhibited growth rates falling between these two extremes. Each point represents mean of 6 tumors. Bars are SD

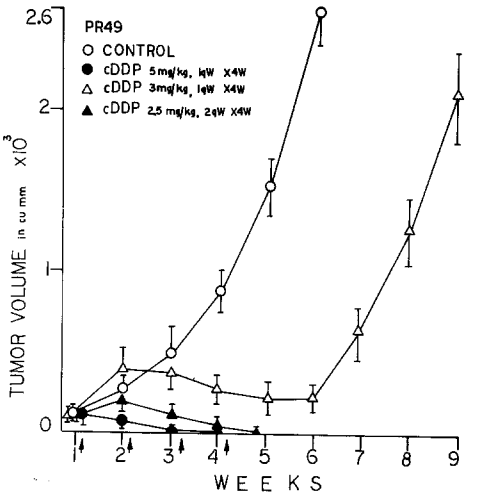

Fig. 3. Response of TCC PR49 to cisplatin using two different dosage levels and treatment schedules. The tumor was highly sensitive to cisplatin when given at the maximum tolerated dose of 5 mg/kg, and fractionated administration of the same amount of cisplatin did not alter tumor response. Cisplatin given as 3 mg/kg once weekly for four weeks resulted only in a temporary arrest of tumor growth. cDDP, cisplatin; 1qW × 4W, once weekly for four weeks; 2qW × 4W, two times weekly for four weeks. Arrows indicate periods of treatment. Each point represents mean of 6 animals. Bars are SD

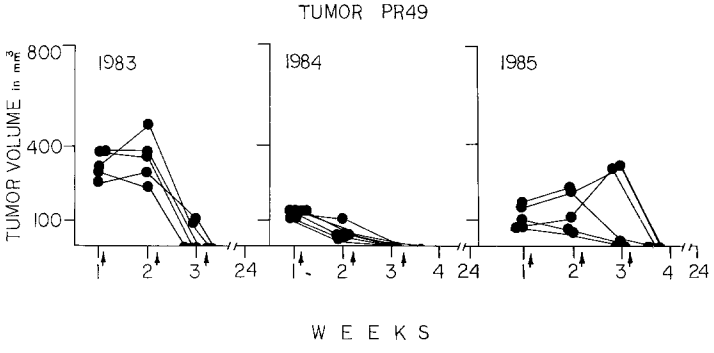

Fig. 2. Response of tumor PR49 to cisplatin at 3 different transplant generations, 5th (1983), 16th (1984), and 27th (1985). Cisplatin was administered as 5 mg/kg once weekly for four weeks. Tumor response was similar regardless of transplant generation. Arrows indicate periods of treatment

carcinomas, covering the entire histopathologic spectrum from the well differentiated to the poorly differentiated neoplasms. While all these tumors have been characterized by a consistent individualized growth pattern through a number of successive transplant generations, considerable variation in growth rates was observed among tumors of the same histogenetic background. When tumor growth rates were compared with tumor histopathologic characteristics, a close relationship was found to exist between these two variables. Figure 1 shows the growth pattern of six TCC grown in the nude mouse. Of these, tumor PR49, a poorly differentiated grade IV TCC, exhibited the fastest growth whereas tumor RT4, a very well differentiated neoplasm, showed the slowest growth rate with tumors of grades II and III falling between these two extremes.

During the course of our studies we were concerned with the question as to whether serially propagated nude mouse-grown human tumors might undergo changes which may alter their reponse to treatment. To answer this question, we selected for testing tumors of known sensitivity to cytotoxic treatment [15, 16]. Figure 2 shows the response of one of them, tumor PR49, to cisplatin over a period of three years (5th, 16th, and 27th transplant generations, respectively). Treatment with cisplatin given as 5 mg/kg once weekly for four consecutive weeks resulted in complete tumor regression between the third and fourth weeks of treatment regardless of both transplant generation and tumor size at initiation of treatment. The same consistency in tumor response behavior was observed with TCC found to be resistant to treatment [2].

Further studies were undertaken to answer two important questions which may influence tumor response: (1) dose of antineoplastic agent(s) needed to achieve maximum response; and (2) tumor behavior in relation to treatment schedule. The results of these studies are summarized in Figs. 3–7.

Figure 3 shows the response of tumor PR49 to cisplatin treatment given at two different dosage levels and treatment schedules. Cisplatin given as 5 mg/kg (maximum tolerated dosage) once weekly for four consecutive weeks resulted in complete tumor regression between the third and fifth weeks from the initiation of treatment. The same tumor response was observed when cisplatin was administered as 2.5 mg/kg twice weekly, both treated groups receiving the same amount of cisplatin within the same time period. In contrast, when cisplatin was given as 3 mg/kg/week for four weeks, only a temporary growth arrest

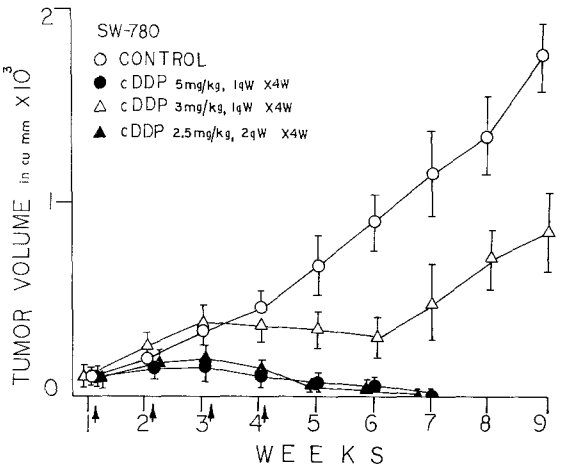

Fig. 4. Response of TCC SW-780 to cisplatin given at two different dose levels and treatment schedules. Compare with Fig. 3. Arrows indicate periods of treatment. Each point represents mean of 6 tumors. Bars are SD. Abbreviations are as in Fig. 3

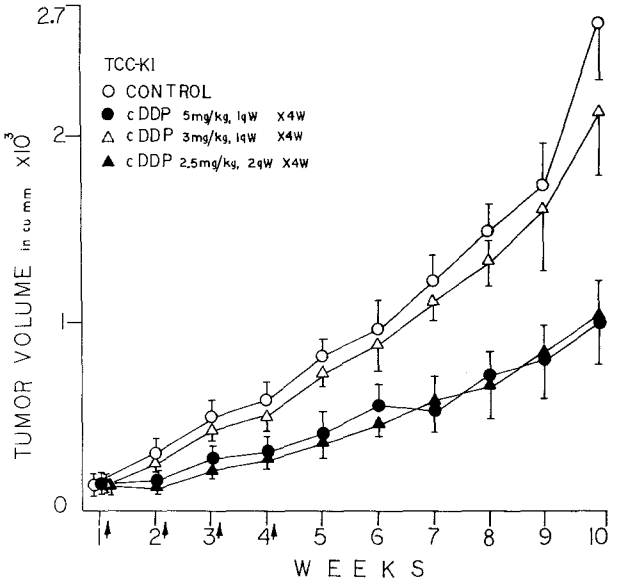

Fig. 5. Response of TCC-K1 to cisplatin showing less sensitivity to treatment. Decrease of cisplatin by 40% (3 mg/kg) had no effect on tumor growth. Arrows indicate periods of treatment. Each point represents mean of 6 tumors. Abbreviations are as in Fig. 3

was observed which was followed by a rapid tumor regrowth. Similar response to cisplatin treatment also was observed with tumor SW-780 (Fig. 4). Dose dependancy of tumor response was found to be more pronounced in tumors showing less sensitivity to cytotoxic treatment. Thus, TCC-K1, a tumor less sensitive to cisplatin, showed no response when cisplatin was given as 3 mg/kg or 4 mg/kg, representing a 40% and 20% dose reduction, respectively (Fig. 5).

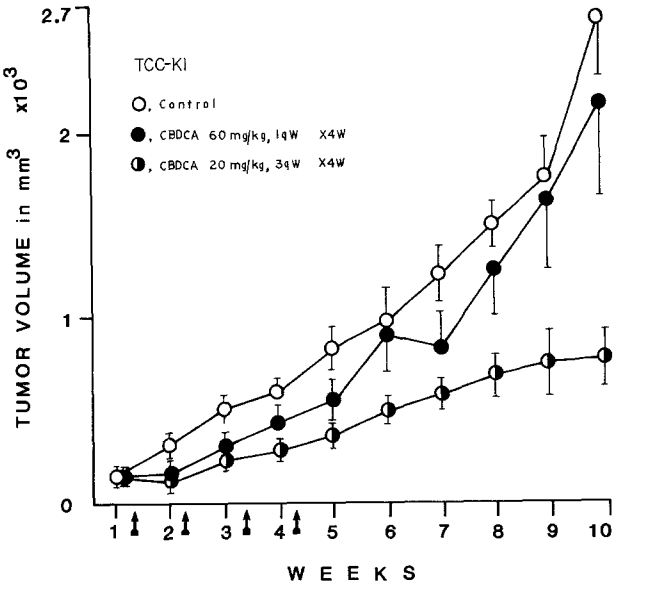

Fig. 6. Response of TCC-K1 to carboplatin given at two different treatment schedules. Tumor response was seen only in the group receiving fractionated carboplatin treatment. Arrows indicate periods of treatments. Each point represents the mean of 6 tumors. Bars are SD. CBDA = carboplatin; treatment abbreviations are as in legend of Fig. 3

The dependency of tumor response on treatment schedule became more evident in experiments using carboplatin. Carboplatin, given in its maximum tolerated weekly dose of 60 mg/kg, had no effect on tumor TCC-K1. When the same amount of carboplatin was fractionated, given three times/week instead of as a single weekly dose, a substantial tumor response was observed characterized by tumor growth arrest and growth delay of many weeks duration Fig. 6). Identical observations were also noted with tumor SW-780 (Fig. 7), and other tumor lines recently tested (unpublished observations). Of note, the antitumor effect of fractionated administration of carboplatin was abolished by decreasing the dose of carboplatin by 30% (14 mg/kg per injection instead of 20 mg/kg).

In an effort to identify additional parameters which may contribute to treatment-related tumor response, the effect of cisplatin on a number of TCC of different grades ranging from the well differentiated grade I to poorly differentiated grade IV tumors was investigated. The relationship between tumor grade and response to treatment is shown in Fig. 8. Tumor PR49 was characterized by complete regression between the third and fourth treatment weeks without tumor regrowth six months later at the termination of the experiment: There was no identifiable tumor at the site of the neoplastic growth at autopsy. Tumor SW-780, a grade III TCC, was equally sensitive to cisplatin treatment which produced complete tumor regression between the seventh and eigth week; however, this short period was followed by tumor regeneration in 50% of the treated animals. Cisplatin was not effective in altering the growth of tumors HBTC1 or RT4, both grade I TCC.

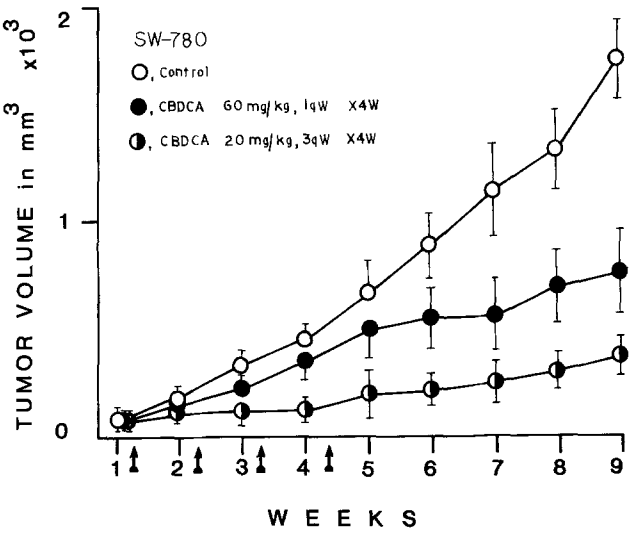

Fig. 7. Response of SW-780 TCC to carboplatin given at two different treatment schedules. Greater tumor response was observed when carboplatin was given in fractionated dosages. Arrows indicate periods of treatments. Each point represents mean of 6 tumors. Bars are SD. Abbreviations as defined in Fig. 3

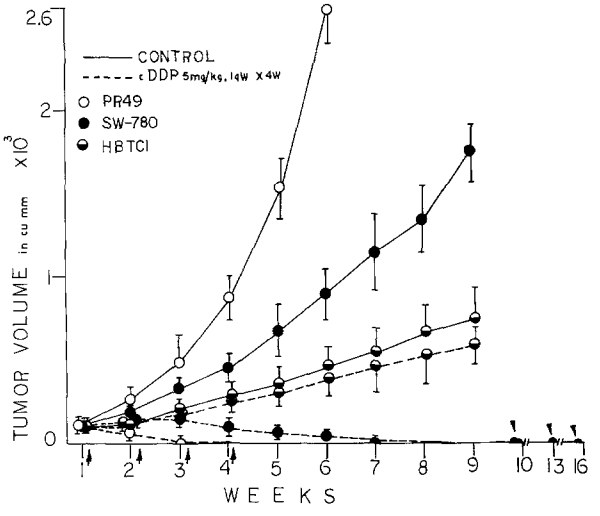

Fig. 8. Tumor response in relation to tumor grade. Tumor PR49, a poorly differentiated TCC, regressed completely following cisplatin treatment without evidence of tumor regeneration. Tumor SW-780, a grade III TCC regressed completely following treatment but regrowth was observed in 50% of the tumors. HBTC1, a grade I TCC, showed no response to cisplatin. Arrows indicate periods of treatment. Arrow heads indicate time of reappearance of tumor after a period of complete regression. Each point represents mean of 6 animals. Bars are SD

Discussion

For a number of years, the biological behavior of nude mouse-grown human malignant tumors of different histogenetic background have been studied with follow up through many successive transplant generations. A remarkable consistency in growth rate characteristics reflecting the degree of tumor differentiation has been found [9, 10, 11, 13], an observation also consistently reported by many investigators. Furthermore, tumor histopathologic characteristics, tumor/host relationship, and tumor-related biological products were faithfully reproduced and could be successfully used in constructing an accurate profile of tumors under study [10, 11, 13]. In addition, the cisplatin-related response studies over a wide range of transplant generations have persuasively indicated that human tumors xenotransplanted in the nude mouse are characterized by biological stability not influenced by the transplant generation at which they are evaluated.

Evaluation of treatment-related tumor response using the nude mouse-human tumor system should consider the important parameters of dose and treatment schedule. The presented data indicate that, regardless of treatment schedule, the dose-response curves are steep [12, 15, 17]. The lack of linearity of dose-response curves is in keeping with the available information from murine tumor systems, various bioassay systems, and clinical studies [4], which emphasizes the importance in selecting the maximum tolerated dose at which level tumor response, if any, should reflect the degree of its sensitivity to treatment.

The present study strongly suggests that treatment schedule is a critical factor in determining antitumor activity. Thus, treatment schedule, timing and sequence of treatments and dose of cytotoxic agent(s) are factors of paramount importance in determining tumor response [12, 14, 15, 16]. In addition, the treatment related difference in the response of tested TCC tumor lines to cisplatin and carboplatin indicate that pharmacokinetic properties should be considered in formulating treatment schedules to assure optimum tissue exposure to agents under evaluation [1–2, 3, 5, 18, 19, 21, 24]. Furthermore, the cisplatin experiments indicate that the degree of tumor differentiation correlates well with tumor responsiveness (Fig. 8), an observation that needs to be evaluated further in additional tumor lines of different histogenetic backgrounds at various grades. Additionally, in all our studies, we closely monitor treatment related histopathological cellular changes, which permit the evaluation of the degree of tumoricidal effect on any experimental procedure thereby making certain that tumor volume changes do not represent a general systemic effect on the host animal [8].

The presented data indicate that nude mouse-grown human tumors are characterized by consistency, reproducibility and biological stability, and permit establishment of human tumor lines which, for tumors of the same histogenetic background, would reflect, if properly selected, tumor heterogeneity as expressed in stage, grade, biological properties, and tumor related biological products. Although each tumor has its own profile, tumors of the same histiotype may be grouped according to commonly shared characteristics thereby leading to a more precise evaluation of factors affecting tumor response to treatment. Such information, if available, may assist the clinical investigator in selecting schedule, timing, sequence and dosages of cytotoxic agents that will permit a more rational design of multidrug and single agent clinical trials.

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